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Perinatal exposure to propylthiouracil (PTU), but not DE-71, induce heterotopia in the brain despite similar effects on serum T4 levels

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Important Dates and Deadlines:

- **May 15, 2020:** Scientific Session/CE Course Proposal Submission Deadline
- **October 9, 2020:** SOT Awards Nomination and Application Deadline
- **October 16, 2020:** 2021 Abstract Submission Deadline
- **October 16, 2020:** Undergraduate Diversity Awards, Perry J. Gehring Diversity Student Travel Award, and Other SOT Undergraduate Awards Deadline

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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1001 SR01: Advances in CRISPR-Cas9 Tools and Applications for Toxicologists

C. Rockwell. *Michigan State University, East Lansing, MI.*

CRISPR-Cas-based technologies have revolutionized science by significantly decreasing the time, money, expertise, and labor required to implement gene editing. Thus, this approach is becoming routine in many laboratories as a facile method to alter the genome. And yet, CRISPR-based methodologies continue to evolve. Recent publications demonstrate that CRISPR gene editing can be expanded in new directions to widen the utility and potential applications of this technology. The first presentation in this course will provide an overview of the latest developments in CRISPR-Cas-based techniques, with a focus on new Cas9 variants with new and expanded capabilities. The first presentation also will cover the new field of RNA targeting and the use of pooled CRISPR libraries with single cell transcriptomics to characterize complex phenotypes. The second presentation will focus on the use of CRISPR-Cas9-based screens from a toxicologist's perspective. Specifically, this presentation will discuss how such screens can be used to gain a detailed, mechanistic understanding of a toxicant's effect as well as the role of specific genes. Overall, this course is designed to provide an overview of the most recent advances in CRISPR-based technologies as well to provide some insight into future uses.

1002 SR02: Qualifying Biomarkers and Navigating the US FDA *Predictive Toxicology Roadmap* to Improve Decision-Making in Toxicology and Drug Safety Assessment

R. Young. *MilliporeSigma, Rockville, MD.*

This session will provide an educational opportunity for attendees interested in learning how information from adverse outcome pathways (AOPs) can be leveraged with two recent US Food and Drug Administration (US FDA) initiatives (i.e., the Biomarker Qualification: Evidentiary Framework and the Predictive Toxicology Roadmap) to improve decision-making for toxicology and human safety issues. The biomarker framework is aimed at identifying and qualifying biomarkers that, within a defined context of use, can be used reliably for specific interpretations and applications. The predictive roadmap is a call to action highlighting the need for a comprehensive strategy to assess new methods and technologies that can improve predictive capabilities, minimize the use of animals, and guide decision-making in regulatory reviews. Because firsthand industry knowledge with these regulatory initiatives may be limited, it is important that experience and learnings available to date be broadly communicated. This session will open with a discussion of ongoing efforts to use AOPs as tools to enhance our mechanistic understanding of various toxicities and to use networks of AOPs as the basis for biomarker development. An example of the genesis and application of biomarkers will be discussed in the context of efforts to develop comprehensive predictions of liver cancer in rodents, including their use in de-risking compounds that would cause cancer through rodent-specific mechanisms. From this initial presentation, session attendees will gain an increased appreciation of the importance of exploiting opportunities for mechanistic screens incorporating biomarkers to define the AOP through which a chemical may mediate effects. The session will then ask a crucial question: After identification of a potentially useful biomarker, what are the next steps needed to ensure regulatory acceptance? To address this point, the second presentation will summarize how the Biomarker Qualification: Evidentiary Framework can be used to define the steps needed to validate the linkage of a biomarker with a specific toxicity or mechanism and provide advice—including a list of dos and don'ts—on how to move efficiently through the process needed to ensure regulatory acceptance of the biomarker for its intended purpose(s). The value of this approach when interacting with regulatory agencies will be demonstrated by reviewing how an understanding of the AOP was used to support arguments that a carcinogenic response with a mutagenic test material could be ascribed to nongenotoxic mechanisms. Collectively, material presented in this session will instill a greater awareness of the value that can be realized with the recent US FDA Biomarker Qualification: Evidentiary Framework and Predictive Toxicology Roadmap initiatives. Although these initiatives were formulated by US FDA, their conceptual underpinnings are universal and applicable for focusing and guiding the dialogue on toxicology issues and concerns with other regulatory agencies, such as the US Environmental Protection Agency. Session attendees will recognize that energetic engagement between industry and regulatory scientists will contribute to the success of the vision of these initiatives and improve decision-making for toxicology, safety assessment, and risk management questions that directly affect human health.

1003 AM03: Developing Therapeutics for Ocular Indications: A 20/20 View

K. Krenzer. *Iuvo BioScience, Rush, NY.*

No longer are eye drops the only way to treat the eye; the emergence of novel therapeutic and development approaches for ocular indications has impacted how we design toxicology assessments, select species, and perform toxicity evaluations. Additionally, the structural complexity of the eye as well as the unique aspects of ocular dose-administration routes require continued refinements of ocular evaluation techniques and assessment strategies. The goal of this course is to provide toxicologists with a broad overview of highly specialized ocular anatomy, current ophthalmic diagnostic techniques, evolving histopathological assessment strategies, and thought-provoking ocular drug development strategies, including case studies. The first speaker will open the session with an detailed overview of ocular anatomy complexity, focusing on unique features of the eye and critical anatomical and physiological features that may influence or impede a drug's efficacy and safety in human patients. The speaker also will discuss novel routes of drug administration in ocular drug development. The second presentation will tune in to gold-standard, in-life ophthalmic diagnostic techniques with selected case examples showing how modern ocular diagnostic equipment can be used to prove multifactorial questions. This presentation also will touch upon recent efforts at harmonization of ocular finding nomenclature and clinical record-keeping lexicon in preparation for SEND (Standard for Exchange of Nonclinical Data) requirements dictating reporting of ocular toxicology studies. The third talk will focus on evolving strategies for histopathological assessment of ocular tissues, which will include key points to consider when processing the eye to ensure a thorough examination of key structures of the globe as well as the effect of factors such as the route of administration and the formulation or character of the therapeutic candidate on effective examination of the eye. The last presentation will highlight unique nonclinical safety challenges and considerations during ocular drug development using case studies to push our assumptions of what is the best way to evaluate the safety of ophthalmic therapeutics.

1004 AM04: Introduction to Open-Access Computational Toxicology Tools

A. Karmaus. *Integrated Laboratory Systems Inc., Durham, NC.*

Computational toxicology is rapidly accelerating our ability to develop methods for predicting chemical properties and chemical-mediated effects, both in the environmental chemical space and in the area of drug development. With frequently updated tools and approaches, overwhelming feedback suggests that more training is needed to help all toxicologists understand the fundamental approaches, use available tools and databases, and interpret outputs. This CE course is designed to offer an introductory-level foundation for leveraging some widely accepted approaches and demonstrate how to use open-source tools and resources to make use of these methods. Course participants across all sectors, ranging from students to career toxicologists, should walk away with the confidence to use the resources presented for computationally characterizing and predicting chemical-elicited toxicity. In addition to gaining familiarity with basic computational toxicology concepts, participants will gain insight into what makes an approach useful for research projects versus which are ready for potential regulatory applications. The first speaker will help lay a foundation for how chemicals are "interpreted" computationally, explaining how chemical structures are leveraged for subsequent analyses (i.e., fingerprinting and its use for read-across). Building on these concepts, the second speaker will provide a thorough example of how to use the US Environmental Protection Agency (US EPA) CompTox Chemicals Dashboard, which provides data for nearly 900,000 chemicals and drugs. Attendees will learn how to assess the confidence in available data, as well as learn how to use the tools available in the dashboard for predicting chemical toxicity and download pertinent data, including mechanistic information, exposure data, animal toxicity doses, and much more. The third presentation will provide a demonstration to empower course attendees in using the Integrated Chemical Environment (ICE), an interactive tool that contains *in vitro* to *in vivo* extrapolation workflows that users can leverage to conduct analyses themselves, as well as provides curated *in vivo* and *in vitro* datasets that can be used to evaluate predicted toxicology outcomes. The fourth presentation will steer the course further into the realm of biological interpretation, describing how toxicogenomics data and literature mining underlying the Comparative Toxicogenomics Database (CTD) can be utilized to computationally characterize chemical mode of action and provide insight into toxicity mechanisms. The last speaker will introduce Sysrev, a collaborative computational systematic review tool to extract pertinent data from literature, incorporating approaches such as machine learning and metadata tagging. Overall, course attendees will gain a fundamental understanding of approaches underlying the most widely used computational toxicology methods, as well as learn to use publicly available, open-source tools that apply these methods.

1005 AM05: Lung Function: It's Not Just Breathing

A. J. Gow. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

There are a variety of techniques available for assessment of the effect of toxicants upon the lung. One of the most powerful techniques available is the measurement of lung function. There have been a number of both conceptual and technical advances made in recent years in the assessment of lung function, particularly with respect to airway dynamics. However, for many toxicologists, lung function assessment is unclear and the possibilities for its use remain underutilized. The purpose of this Continuing Education course is to examine the fundamentals that underlie lung function testing and consider in what ways they can be measured and how these data can be related to toxicological outcomes. The first presentation will introduce the concept of lung function and how it has developed with newer technologies, in particular relating structure with function and how modeling can play a part in assessing this relationship. The second presentation will present an overview of lung mechanics. The physiological elements that make up the measurable units of lung function and how pressure and flow data can be used to generate models of lung function to assess toxicological outcomes will be discussed. The third presenter will describe the challenges of measuring lung function and the various approaches that are available to the investigator. The presentation will concentrate on parameters that can be extracted from different measurement techniques and how they can be utilized to gain information about the physiological elements of the lung. This information will be of critical importance to the practicing toxicologist. The fourth presenter will present on feed forward modeling of lung function and how both pathological and physiological data can be combined. He will focus on how animal scale function data can be used to understand the consequences of toxicant exposure to humans. The final presenter will provide a detailed example of how imaging data can be used to predict lung function. The focus of the presentation will be the longitudinal analysis of whole animal *in vivo* imaging data following toxicant exposure and its relationship to outcome. This technique provides a novel paradigm for assessing the continuous effect of toxicological exposure in the lung. Overall, these presentations will provide the audience with a practical understanding of lung functional data within the context of disease models as well as an understanding of how lung functional data can be used to further their own research.

1006 AM06: Modern Modeling Strategies to Address Uncertainty and Variability in Dose-Response Assessment

K. Shao. *Indiana University, Bloomington, IN.*

Quantifying dose-response relationships to evaluate the toxicity of environmental chemicals is a key step in human health risk assessment and has evolved substantially in recent years. In addtion to fundamentally developing a dose-response curve and estimating a dose level that results in a predetermined critical effect, recent advances in toxicology and modeling stategies enable dose-response assessment to more comprehensively and quantitatively address uncertainty and human variability. The purpose of this course, to be delivered by a mixed group of experts from academia, government, and industry, is to provide participants an overview of the cutting-edge modeling strategies employed in dose-response assessment to quantify uncertainty and variability. The first presentation will introduce the benchmark dose (BMD) methodology and its utilities to quantify various sources of uncertainty in dose-response modeling with a demonstration of the Bayesian BMD modeling system. The second speaker will present an overview of the principles and recent applications of probabilistic dose-response assessment approaches developed under the WHO/IPCS guidance to address uncertainty and variability in quantitative risk assessment. The third presentation will provide an overview together with case examples of Diversity Outbred (DO) mouse population-based *in vitro* systems to demonstrate a data-driven probabilistic approach to derive a chemical-specific uncertainty factor for inter-individual variability. The last speaker will introduce how to predict population distributions of toxicokinetic-relevant physiological quantities that NHANES does not measure based on the measured counterparts using the HTTK-Pop R package that incorporates population variability in high-throughput toxicokinetic modeling. Throughout the course, use of and applications to *in vitro* and high-throughput testing systems will be highlighted, including their relevance to *in vitro* to *in vivo* extrapolation (IVIVE).

1007 AM07: The Basics of *In Vitro* Xenobiotic Metabolism and Drug-Drug Interaction Investigations: Applicability to All Xenobiotics

A. Parkinson. *XPD Consulting, Shawnee, KS.*

In the current landscape of drug development and investigation of environmental chemicals, many personnel with toxicology backgrounds find themselves overseeing not only preclinical safety assessments but also *in vitro* xenobiotic metabolism and interaction studies. Therefore, the goal of this course is to provide attendees with practical perspectives from experts in the field on the approaches and techniques that are available to address the important aspects of xenobiotic metabolism. The first presentation will focus on the experimental design and interpretation of data obtained from studies of *in vitro* inhibition of cytochrome P450 (CYP) and other xenobiotic-metabolizing enzymes. In contrast, the second presentation will focus on the experimental design and interpretation of data obtained from studies of *in vitro* induction of CYP and other xenobiotic-metabolizing enzymes. The third talk will broaden considerations of xenobiotic metabolism, including assessment of the toxicological burden of reactive metabolites, and the experimental design and interpretation of data obtained from studies of *in vitro* reaction phenotyping techniques in human liver microsomes and other test systems derived from humans or toxicologically relevant species. The fourth presentation will discuss *in vitro* approaches for determining the potential for xenobiotics to be substrates or inhibitors of major transporters that are of regulatory interest and interpretation of the data obtained from these studies. The final presentation will discuss the practical implications of the *in vitro* approaches discussed and the current regulatory thinking on their applicability to all xenobiotics to which humans are exposed. The overarching objective of this course is to provide attendees with a solid foundation in commonly used methods to enable the design, execution, and interpretation of rigorous and reproducible *in vitro* xenobiotic metabolism and interaction studies that will withstand regulatory scrutiny and avoid common pitfalls. This course will benefit those whose toxicological interests are expanding to include *in vitro* xenobiotic metabolism, as well as professionals responsible for appraising or evaluating *in vitro* xenobiotic metabolism, interaction studies, and other ADME data for internal decision-making, risk assessment, or submission to various regulatory agencies.

1008 AM08: Timing is Everything: Developmental Exposure Alters the Path of Immune Cell Maturation and Function

A. Venosa. *University of Utah, Salt Lake City, UT.*

There is compelling evidence that exposure to exogenous agents at different stages of development contributes to disease later in life (and across generations), with animal models supporting this concept in reproductive, metabolic, and neurodegenerative diseases. Two major notions link prenatal and early-life exposure to increased risk of disease later in life—namely, the Barker hypothesis and the hygiene hypothesis—while epigenetic reprogramming may extend this susceptibility across future generations. The immune system represents a unique niche of cells tightly entangled with the parenchyma in every tissue, acting as pro-homeostatic sentinels equipped to mount the appropriate response upon exogenous aggression. While the linkage between developmental immunotoxicity (DIT) and susceptibility to later-life diseases is an accepted paradigm, the mechanisms by which exogenous agents impact the developing immune system and change disease susceptibility are not well established. Clinical evidence suggests that the underlying effects of these agents may be masked until triggered by a later-life event (i.e., infectious exposure or aging itself), at which point the immune response may enact a super-responsive state, favoring disease pathogenesis. Recent evidence highlights myriad variables to take into account to accurately study DIT, including the importance of evaluating the appropriate window of vulnerability; establishing whether the toxicant exerts direct and long-lasting effects on the immunological machinery or reprograms the behavior of bystander parenchymal cells; outlining which pathway each environmental agent will be affecting; and, more recently, determining sex-based outcomes to exposure. With basic and translational researchers facing the challenge of elucidating the molecular mechanisms mediating DIT, it is pivotal that regulatory agencies and industry work in unison toward implementing safety protocols that address these factors. Therefore, this Continuing Education course proposes to (1) inform the attendees on the current advances in the design and execution of DIT studies geared at developing preclinical tools to predict risk of adult-life disease; (2) provide the most recent evidence, spanning multiple phylogenetic species (nonhuman primates, rodents, and fish), of DIT across a wide array of exogenous agents; and (3) provide insights on the impact that studying DIT could provide at the regulatory level.

1009 PM09: AAV Vectors on the Move: Safety Assessment and Advancing Sciences in Gene Therapy Second-Generation Products

S. Korte. *Covance Preclinical Services GmbH, Münster, Germany.*

The field of gene therapy has matured in recent years to bring forward a potential treatment or cure for life-threatening monogenetic diseases. The first successful treatments are already available to patients in various indications, and even more are undergoing active preclinical and clinical development. This CE course aims to summarize the great achievements already established for this promising therapeutic modality, will provide insights into the specific considerations for meaningful preclinical safety assessments, and will introduce the regulatory framework, as well as looking into advancements in the field opening up new opportunities and challenges. This CE course will start with a review of the history of adeno-associated virus (AAV) vector development and give a current status of its clinical use and challenges. A number of unique considerations need to be addressed when designing a nonclinical safety assessment program for AAV gene therapies (GTs), including the off-target tissue distribution and transgene expression, persistence, and immunogenicity. Additionally, although AAVs are generally considered nonintegrating, the potential implication, if any, of a low frequency of integration is not yet well understood. Attendees will hear an overview of preclinical safety assessment strategies for AAV gene-based therapies, including dose extrapolation for first-in-human starting dose, and learn about AAV screening approaches in nonhuman primates (NHPs) and case studies of regulatory-accepted toxicity studies via the oral, IV, and subretinal routes. In addition, a case study of the biodistribution and toxicity profile of an occular AAV gene therapy administered by intravitreal injections (IVT) will be shared. The course will expand into questions on impurity profiling of the drug product and conclude with an overview of the regulatory guidelines for GTs and the sharing of regulatory experience in the field. Take advantage of the renowned group of speakers coming together in this course to share their experience in and deep insight of the field of gene therapy with safety specialists from academia and the pharmaceutical industry, as well as regulatory risk assessment experts. This course offers a rare chance to receive guidance in this emerging and promising scientific area and learn about and discuss its particular challenges.

1010 PM10: An Introduction to New Approach Methodologies (NAMs) and Understanding Their Potential to Support Regulatory Decisions

M. Krishan. *Danone North America, Louisville, CO.*

Recent shifts in the global regulatory landscape to consider the use of nonanimal testing methods have led to significant advances in the development of alternative test methods to replace, reduce, and refine animal use. The term *new approach methodologies* (NAMs) broadly describes any nonanimal technology, methodology, approach, or combination that can be used to provide information on chemical hazard and risk assessment. With new opportunities comes new challenges, such as validation of test results, understanding their applicability in different sectors and risk assessments, and global regulatory acceptance of these methods. Despite these challenges, the development, use, and acceptance of these predictive toxicology methods is on the rise. There is a wealth of knowledge and data that is being generated with NAMs; however, there are questions on when, how, and where can we use these NAMs. This CE course will provide an overview of NAMs along with case studies where they are being used or could potentially be used for regulatory risk assessment. The speakers will present on (1) chemical-biological data and analysis tools (Tox21/ToxCast) and examples where high-throughput screening (HTS) methods have been approved for use in regulatory decision-making; (2) read-across approaches and their use in regulatory risk assessment; (3) use of evidence maps and systematic reviews and case studies with a focus on application of each to regulatory risk assessment; (4) use of the adverse outcome pathway (AOP) including most well-developed examples of AOP-supported decision processes for evaluating skin sensitizing potential and a computational model to predict the likelihood of reproductive impairment based on aromatase inhibition; and (5) concepts underpinning Integrated Approaches to Testing and Assessment (IATA) and concrete examples for assessing developmental neurotoxicity (DNT) and carcinogenicity of chemicals used in a variety of sectors. Also, updates will be provided on IATA case study projects currently running at the OECD and a set of resources being developed to support IATA development, evaluation, and regulatory uptake. This course will be useful to those interested in understanding the regulatory application of NAMs.

1011 PM11: Gateway Technologies to Tomorrow's Metal Toxicological Research

W. Zheng. *Purdue University School of Health Sciences, West Lafayette, IN.*

Infotechnology and biotechnology represent two leading technological breakthroughs underpinning the future advances in medical science and human health. Big data algorithms not only offer unique advantages by the machine learning for fast processing of existing data, but more importantly, through learning, they maximize the chances of successful choices and adaptations for accurate analysis of cumulative toxicological data, prediction of flexible outcomes, and influences in policy decisions. The CRISPR technology, on the other hand, allows impeccable gene editing that has already transformed biology and genetics, lending itself to an effective, precise, and cheap method for mechanistic investigations. Applying CRISPR facilitates our understanding of the events underlying xenobiotics' cellular and molecular interactions. However, application of both artificial intelligence (AI) and CRISPR in metal toxicological studies remains in its infancy. For metal quantitation, recent advances in specific fluorescent metal-binding ligands render it feasible to trace the subcellular trafficking of interested metals through live-time cell imaging. Moreover, a variety of animal models for metal toxicity evaluations have been developed in the past several decades, ranging from *Drosophila*, *C. elegans*, and zebrafish to rodents and nonhuman primates. How to choose the right species and animal model(s) for a particular study of metal toxicity represents a new challenge. This advanced course is designed to introduce the audience with novel concepts and technologies in metal toxicological research. The first presentation will briefly review the history of metal toxicology within the context of technology advancement, followed by identifing gaps in the field and illustrating the impact of emerging technologies on the future direction of metal research. The second presentation will focus on the assessment of metals in cellular models and tissues; the speaker will showcase how fluorescent reporters, advanced imaging and spectroscopy, and genetic- and protein-based biomarkers can be used to monitor tissue and cellular distribution of metals. The third presentation will go further toward the precise mechanistic study of metal toxicities; the speaker will provide the overview of CRISPR technology and will cover procedures for investigations of metal-induced neurotoxicities. The fourth presentation will focus on the framework for choosing the most informative animal model to study modes of chemical toxicities, neurotoxic risks, and therapeutic treatment. Finally, the last presentation will introduce the concept and general practice of AI in health research, followed by integrative examples of how to use AI to interpret chemical toxicities as well as the policy regulation. Speakers will discuss these concepts and technologies with details specific to metals having particular human, environmental, and occupational health relevance, such as lead (Pb), manganese (Mn), cadmium (Cd), arsenic (As), silver (Ag), and mercury (Hg). The course will benefit those who desire a learning of advanced technologies for mechanistic interpretation and machine-assisted prediction of metal or chemical toxicities, and technical approaches in utilizing widely available CRISPR and cellular imaging technologies that can be used to support research in metal toxicology. As the course introduces concepts and techniques that are equally applicable to other fields, such as neurotoxicology, nanotoxicology, carcinogenesis, risk assessment, and occupational health, researchers engaged in these wider aspects of the toxicological sciences will benefit by attending this course and acquiring knowledge beyond metals.

1012 PM12: Harnessing the T Cell for Cancer Immunotherapy: A Course on T Cell Redirection

J. L. Lynch. *Janssen Research & Development, Spring House, PA.*

Cancer immunotherapy is an area that has been of great interest in the last few years as several new therapeutic approaches have shown encouraging results in the clinic and subsequent approvals. The normal immune system has a protective capacity against tumor cells (immunosurveillance), while tumors can employ mechanisms that can result in an insufficient supply of activated and/or antigen-specific T cells within the microenvironment (tumor evasion). To overcome this immune-evasive mechanism, T cells can be redirected and expanded within the tumor microenvironment. CD3 redirection, which leverages protein-based therapeutics to simultaneously bind CD3 on T cells and a tumor associated antigen (TAA) on tumor cells, and engineered T cell-based therapeutics to redirect T cells to TAAs are emerging as powerful ways to harness the immune system to combat malignancies.The goal of this course is to provide the investigator with an overview of T cell redirection technologies and how to design a nonclinical safety strategy to understand safety liabilities. An overview of chimeric antigen receptor (CAR) T cells, T cell receptor (TCR) T cells, CD3 bispecifics, and immune mobilizing monoclonal TCRs against cancer (ImmTac) modalities will be provided. In addition, successful nonclinical safety strategies to support first-in-human clinical trials for

these modalities will be shared. Overall, this course will provide a comprehensive overview of T cell redirection platforms, study design, and the challenges associated with these modalities.

1013 PM13: *In Vitro* Approaches to Assess the Toxicity of Inhaled Substances

A. Clippinger. *PETA International Science Consortium Ltd., London, United Kingdom.*

Inhalation is a major route of human exposure to airborne substances, and as such, there are regulatory and nonregulatory needs to assess the potential toxicity of inhaled substances. While the standard regulatory requirement is a rat inhalation toxicity test, anatomical and physiological differences between rodents and humans have led to substantial investment in the optimization of alternative approaches. These alternative approaches can be based on human mechanisms of toxicity, thus better protecting human health while reducing animal use. In this course, speakers from government, contract research organizations, academia, and NGOs, as well as method developers, will discuss progress and challenges associated with various approaches for inhalation toxicity testing. It will include an introductory overview, setting the stage for the remaining talks by discussing the currently used rat inhalation tests and how an alternative approach can be demonstrated to be a valid replacement. Other topics to be covered will be the use of cell culture systems, 3D reconstructed human tissue models, and human precision-cut lung slices, as well as the use of *in vitro* exposure devices for deposition of test chemicals. The final speaker will present a regulatory perspective on processes in place that allow for acceptance of alternative approaches for inhalation toxicity testing, highlighting a successful example. These presentations will explore the value of the air-liquid interface (ALI) for testing, advantages and limitations of different approaches, and case studies of the use of different model systems in both nonregulatory and regulatory paradigms. Overall, a course attendee should learn about the state-of-the-science of *in vitro* approaches for respiratory toxicity testing and gain insight into determining which method is most appropriate, depending on the test substance and purpose of the study. This course is aimed at scientists at all levels from industry, government, and academia.

1014 PM14: The Male Reproductive Tract: Development, Toxicology, and Pathology

V. Sutherland. *NIEHS/NTP, Research Triangle Park, NC.*

The male reproductive system develops *in utero*—in rats during mid-gestation and in humans during the second month of pregnancy—but does not fully mature until puberty. Exposure to xenobiotics (e.g., diethylstilbestrol and phthalate exposure) during any stage, particularly during development and maturation, can adversely affect a male's reproductive potential and play a significant role in development of a diseased state. Understanding what normally happens at these critical stages of development can lend clues to determine when an exposure has happened, what tissues are affected, and if functional capabilities will be impacted. Defining potential effects is routinely performed with guideline reproductive and developmental experiments and in academia with focused studies; however, these assessments do not always include histopathology evaluations, and if they do, the rigor needed for select tissues may not be utilized. Inclusion of histopathology, especially during select stages, may help identify a pattern of toxicities, subtle effects of an endocrine-disrupting chemical, or lesions that can lead to future reproductive issues (e.g., infertility). This additional data can expand our capabilities in characterizing potential modes of action that result in functional changes. Thus, a field that did not routinely assess tissues in more than a functional manner is now exploring the utility of pathology evaluations at stages not previously studied (e.g., juvenile assessment of cell populations in the testes) and appreciating that these tools can assist in recognizing patterns of toxicity. Therefore, a full toxicological and histopathology assessment of the male reproductive tract may provide additional information on functional effects, assist in determining which part of the system was targeted and how to mitigate concerns, and, for select issues, provide an early read on potential problems. This course will cover development and maturation of the male reproductive tract, explaining impacts on function at different time periods (*in utero*, juvenile, and adult) and addressing the potential value of histopathology at both the juvenile and the adult stages. Case studies will be used to highlight the toxicological significance of the effects of xenobiotics on male reproductive system toxicity. Understanding patterns of toxicity (e.g., effects in organ weight linked with findings in other tissues or pathology findings observed in a young animal correlating to outcomes in an adult) and utilizing some of the newer techniques and protocols (e.g., fetal testis explants, biomarkers) will not only provide a better understanding of what endpoints are affected but also may provide us with the tools to design better studies and correlate findings at earlier stages with long-term functional effects. To this end, four speakers, each a world-recognized expert in male anatomy, development, reproduction, and/or pathology, will discuss functional assessments of the male reproductive tract and address the utility of pathology in male reproductive and development evaluations.

1015 Can We Predict and Manage Immune-Related Adverse Events Associated with Cancer Immunotherapy?

Y. Yoshioka. *Osaka University, Osaka, Japan.*

Cancer is a major disease burden worldwide, with considerable impact on society. For several years, there has been intense excitement concerning immunotherapy against cancer. Immunotherapy takes many forms in cancer treatment, including the adoptive transfer of *ex vivo* activated T cells (T cell adoptive immunotherapy) and administration of antibodies that block the so-called immune checkpoint pathways. The recent success of immune checkpoint inhibitors, such as monoclonal antibody blocking of cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD1) or its ligand, programmed cell death ligand 1 (PD-L1), has boosted the development of cancer immunotherapy. The Nobel Prize in Medicine 2018 was awarded to Dr. James P. Allison and Dr. Tasuku Honjo for their work on immune checkpoint inhibitors as a new cancer therapy. Although remarkable clinical responses have been observed in cancer immunotherapy, these therapies often have inflammatory side effects leading to immune-related adverse events (irAEs). Immune-related adverse events have occurred with a high frequency in many cases. Although any organ system can be affected, irAEs most commonly involve the gastrointestinal tract, endocrine glands, skin, and liver. The wide range of irAEs associated with cancer immunotherapy can complicate this effective therapy and limit its use in patients with cancer. While the adverse events were controllable in some cases, severely deleterious outcomes (including death) have been reported. The mechanism of toxicity may vary according to the immunotherapy, although the precise pathophysiology underlying irAEs is unknown. Understanding the mechanisms of irAEs is needed to predict and manage these toxicities, which would include identification and validation of reliable surrogate biomarkers. The goal of this Symposium is to present experts who will speak on key topics related to irAEs associated with cancer immunotherapy. The specific topics that will be covered include (1) mode of action of checkpoint inhibitors, immunologic biomarkers, and clinical responses; (2) the potential of autoantibodies as biomarkers for predicting risk to develop autoimmune diseases following treatment with immune checkpoint inhibitors; (3) management of irAEs induced by T cell adoptive immunotherapy; and (4) clinical success of immune checkpoint inhibitors that received market authorization—and current challenges to maximize the efficacy of immune checkpoint blockade and combination therapies. This Symposium containing cutting-edge information will be of interest to many SOT members, including those from the Immunotoxicology, Regulatory and Safety Evaluation, and Clinical and Translational Toxicology Specialty Sections, as well as to other participants from academia, industry, and government regulatory/safety agencies. This Symposium represents another in the continuing annual collaborative sessions co-chaired/organized by the Japanese Society of Immunotoxicology (JSIT) and the SOT Immunotoxicology Specialty Section.

1016 Mode of Action of Checkpoint Inhibitors, Immunologic Biomarkers, and Clinical Responses

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Although the immune system plays a critical role in controlling and eradicating cancer, cancer has developed multiple mechanisms to suppress the antitumor immune responses and evade its wrath, including production of inhibitory cytokines, recruitment of immunosuppressive immune cells, and upregulation of coinhibitory receptors known as immune checkpoints. Recent approvals of monoclonal antibodies targeting two immune checkpoints, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death protein 1 pathway (PD-1/PD-L1), as well as encouraging data emerging from clinical trials, have demonstrated durable responses against a wide array of tumor types and are transforming the way cancer is being treated. However, releasing the breaks of the immune system also can lead to an immune response that is directed not at the cancer but at normal tissue, leading to immune-related adverse events. This lecture will discuss the biological rationale and clinical experience with these checkpoint inhibitors. Furthermore, while there have been many successes, there also continues to be failures, and thus strategies to increase their effectiveness while managing their safety, such as combinations and biomarkers for patient selection, will be discussed.

1017 Autoantibodies as Biomarkers for Predicting Risk to Develop Autoimmune Diseases Following Treatment with Immune Checkpoint Inhibitors

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Immune checkpoint inhibitors (ICI), such as antibodies to PD-1/PD-L1, are a new type of cancer immunotherapy. Because ICI enhance tumor immunity, development of autoimmune disease has been reported among the adverse events. However, biomarkers for predicting risk to develop autoimmune diseases remain to be established. Autoimmune thyroid disease is a common autoimmune condition seen in 3%-5% of the general population; thus, subclinical thyroid diseases would not be unusual in patients with cancer. Analysis of autoantibodies in patients with lung cancer revealed that ~15% of them have known autoantibodies of systemic autoimmune rheumatic diseases. Because production of autoantibodies usually precedes clinical manifestation, it may be reasonable to predict that the presence of autoantibodies is associated with the risk to develop autoimmune diseases. Autoantibodies associated with autoimmune thyroid disease and systemic rheumatic diseases were evaluated in lung cancer patients receiving ICI before and during ICI therapy. Thyroid autoantibodies were tested by enzyme-linked immunosorbent assay (ELISA) for antibodies to thyroglobulin and thyroid peroxidase. Autoantibodies associated with systemic autoimmune diseases were examined by ELISA (Ro60, Ro52, Jo-1, CENP-A, CENP-B) and radioimmunoprecipitation using 35S-methionine labeled K562 cells. Among nine cases treated with ICI, three developed hypothyroidism. Two of them had thyroid autoantibodies before ICI therapy and one case developed them after ICI. Six cases without hypothyroidism did not have thyroid autoantibodies before or after ICI therapy, suggesting thyroid autoantibodies are associated with hypothyroidism during ICI and have predictive value. Five cases developed interstitial lung disease (ILD) during ICI therapy. Two of them had anti-Ro52 before ICI and one developed anti-Ro52 followed by ILD. Anti-Ro52 found in ~0.5% of healthy individuals and 11% of lung cancer patients may be associated with the risk to develop ILD after ICI therapy. These results suggest that detection of autoantibodies associated with corresponding autoimmune disease may help in predicting the risk to develop autoimmune diseases.

1018 Immune-Related Adverse Events Induced by T Cell Adoptive Immunotherapy Using Genetically Engineered TCR-T Cells

H. Ikeda. *Nagasaki University, Nagasaki, Japan.* Sponsor: Y. Yoshioka

It is becoming increasingly clear that T cell adoptive immunotherapy with genetically engineered T cells, such as CAR-T therapy and TCR-T therapy, has the potential to control and even cure cancer in some patients. On the other hand, severe irAEs associated with efficacy have frequently been reported in clinical trials. In some cases, the irAE could be attributed to T cell reactivity against normal tissue where the target protein was expressed. Furthermore, on-target irAE such as cytokine release syndrome (CRS) as a consequence of producing supraphysiologic levels of cytokines by engineered T cells upon antigen recognition, and immune effector cells associated neurotoxic syndromes (ICANs), pose a major challenge to this type of therapy. The irAE induced in a phase I clinical trial of T cell adoptive immunotherapy utilizing genetically engineered to express tumor-reactive TCR genes for the treatment of patients with advanced solid tumors will be discussed. A strategy to utilize allogeneic T cells with reduced GVHD toxicity also will be presented.

1019 Nonclincial Paths for Immunomodulators: Current Challenges to Evaluate the Safety of Immune Checkpoint Inhibitors and Combination Therapies

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Checkpoint inhibitors have created a drastic shift in the immunotoxicology field. Prior immunotoxicology assessments were designed for small molecules and centered on immunosuppression. With the advent of immunomodulators for patients with serious and life-threatening disease, this created a unique safety evaluation challenge. Existing nonclinical models were not especially helpful, as the immunomodulatory antibodies were not very active in those models due to species specificity as well as the lack of any immunostimulatory, pathogenic signal in the animals that was present in the patient population. These unique safety challenges required a flexible approach with a combination of *in vitro* testing and consideration of the immune pathway being altered (which can help predict effects in humans), as well as strict safety monitoring of the patient population. Although there have been a few unexpected adverse events noted with combination therapies, the data to date support a flexible approach that allows for an adequate safety evaluation for products intended for patients with serious and life-threatening illnesses.

1020 Developmental Toxicity of Per- and Polyfluoroalkyl Substances (PFAS): Current *In Vivo* Approaches and Application to Human Health Risk Assessment

J. Conley. *US EPA, Research Triangle Park, NC.*

Per- and polyfluoroalkyl substances (PFAS) hazard and risk assessments are a high-priority focus of health-based agencies on a global scale due to widespread occurrence and exposure. The PFAS chemical class is diverse, in regard to physicochemical and toxicokinetic characteristics, and extensive, with hundreds to thousands of existing structures and additional novel structures introduced annually. The legacy PFAS, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), have been studied extensively in traditional and alternative animal models; however, little to no data regarding persistence, toxicokinetics, or toxicity are available in the peer-reviewed literature on emerging PFAS with known human exposures, such as the perfluoroalkyl ether acids (e.g., hexafluoropropylene oxide-dimer acid [GenX] or Nafion byproduct 2). Importantly, the specific molecular mechanisms and downstream events responsible for the adverse effects described in rodent studies of PFOS and PFOA, including neonatal mortality, immunomodulation, altered thyroid hormone concentrations, and impaired carbohydrate and lipid metabolism, are relatively unresolved. Further, it is unknown if exposure to emerging PFAS elicit similar developmental outcomes, and how their relative potencies compare with those of legacy PFAS. Thus, research examining critical upstream events, as well as previously unstudied adverse outcomes from early-life exposures, is needed to facilitate screening of PFAS and inform human health and environmental risk assessments. A key assumption has been that many, if not all, of the adverse effects of PFAS are mediated in large part by modulation of peroxisome proliferator activated receptors (PPARs), particularly PPAR-alpha. However, it appears that multiple molecular mechanisms may be involved in the spectrum of effects described for developmental PFAS toxicity. The first three presentations in this session will focus on *in utero* and early life stage exposure studies in laboratory rats and mice to emerging and legacy PFAS. Research presented will cover the current state-of-the-science for *in vivo* effects on neonatal viability, neurological responses to alterations in thyroid hormone concentrations, development of immune competence, and metabolic capacity. Each presentation will pay particular attention to elucidating the specific molecular mechanisms and key events associated with the adverse effects of PFAS exposure. The final two speakers of the session will present current state-level (US) and international (European Union—European Food Safety Authority) regulatory approaches for derivation of human health-based guidance levels. These presentations will include a case study of the utilization of *in vivo* toxicity data for generating a transgenerational exposure model to address critical life stages for PFOA, and the use of epidemiological studies and the potential for incorporating a mixtures-based approach to characterize risk of multiple PFAS in food.

1021 PFHxS and Developmental Neurotoxicity: Does Thyroid Hormone Action Play a Role?

K. O'Shaughnessy. *US EPA, Research Triangle Park, NC.*

Perfluorohexane sulfonate (PFHxS) is an environmental thyroid-disrupting chemical that reduces serum thyroid hormones (THs) in animal models. As thyroid action is required for normal brain development, it is suspected that PFHxS may induce developmental neurotoxicity by endocrine-mediated mechanisms. Using a multidisciplinary approach, we investigate whether PFHxS induces abnormal brain development and function associated with TH dysregulation. Pregnant rats were orally dosed (50 mg/kg/day) from gestational day 6 (GD6) to postnatal day 21 (PN21), and the offspring analyzed. Results show that PFHxS exposure reduced serum THs in both the dams and pups. Serum total thyroxine (T4) was reduced by approximately 75% in exposed neonates. However, remarkably, brain T4 was significantly reduced in the pup brain only on PN0, and not on PN2, PN6, or PN14. Brain triiodothyronine (T3) also was not significantly reduced at any stage tested. We also detected no evidence, transcriptionally or phenotypically, of TH-mediated dysfunction of the cortex. No significant differences in learning and memory were detected in offspring aged to adulthood, as assayed by trace fear conditioning; sensory gating was similarly unaffected. These data suggest that despite significant reductions in serum T4, the neonatal rat brain does not appear to be TH insufficient following PFHxS exposure. These observations may be attributed to chemical action: PFHxS purportedly reduces serum THs by interfering with the function of serum binding proteins. However, unbound

(free) THs are transported across the blood-brain barrier and into the tissue. Thus, these dynamics in hormone transport may explain why brain TH concentrations are largely unaffected by PFHxS exposure at the stages tested. This hypothesis is consistent with clinical presentations of patients and knockout mice possessing loss of function mutations in serum binding proteins. In the future, additional work is warranted to determine if other perfluorinated compounds alter brain TH concentrations at other developmental stages and/or induce abnormal brain development by other mechanisms. *This work does not reflect US EPA policy.*

S 1022 Adverse Developmental Effects of Gestational Exposure to Emerging Perfluoroalkyl Ether Acids: Mechanistic Insights and Potency Comparisons with Legacy PFAS

J. Conley. *US EPA, Research Triangle Park, NC.*

Perfluoroalkyl ether acids (PFEAs) are a subclass of PFAS and are currently used in the production of fluoropolymers following the phase-out of the legacy PFAS perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). PFEA compounds such as hexafluoropropylene oxide dimer acid (GenX), Nafion byproduct 2 (NBP2), and perfluoro-2-methoxyacetic acid (PFMOAA) have been detected in surface water, drinking water, and/or human serum, yet little to no published toxicity data are available. We previously determined that oral GenX exposure to pregnant Sprague-Dawley rats produced adverse maternal and F1 effects similar to those produced by PFOS, but with lower potency. Here, we assessed the *in utero* toxicity of PFMOAA (0.01-200 mg/kg/d, gestation day [GD] 14-18 and GD 9-13) and NBP2 (0.1-30 mg/kg/d from GD 14-18 and 0.3-30 mg/kg/d from GD 8-postnatal day [PND] 2) for comparison with GenX and the legacy PFAS. PFMOAA was negative for adverse maternal and F1 effects; however, NBP2 reduced maternal weight gain (GD 14-18 and GD8-PND2 dosing), reduced neonatal survival (=10 mg/kg), and reduced pup weight (GD8-PND2 dosing). Based on oral ED_{50}s for neonatal mortality, NBP2 was only ~3-fold less potent than PFOS (9.5 mg/kg for NBP2 versus 3.1 mg/kg for PFOS), whereas GenX was ~35-fold less potent than PFOS. It appears that the spectrum of adverse developmental effects is similar between some of the PFEAs and the legacy PFAS and that the oral potency is also relatively similar for some compounds. Data from our GenX studies indicated that many genes associated with glucose metabolism were significantly downregulated in PND 0 pup livers and impaired liver glycogen deposition of the developing fetus/neonate may be a principle key event for reducing F1 survival and body weight. Ongoing research in our group is investigating the putative mechanism(s) of action (primarily activation of peroxisome proliferator-activated receptor subtypes) and additional key events that lead to adverse maternal and neonatal outcomes, comparison of internal dosimetry for extrapolation with human exposures, and mixture-based effects of *in utero* exposure to multiple PFAS. *Abstract does not necessarily reflect US EPA policy.*

S 1023 Developing an Understanding of the Effects of PFAS on the Immature Immune System

J. DeWitt. *East Carolina University, Greenville, NC.*

Studies of children who have been exposed to PFAS throughout fetal and early life have reported reduced responses to common childhood vaccines. Studies in experimental rodents exposed to PFAS also demonstrate that exposed rodents have a reduced ability to mount an appropriate immune response against novel antigens. The ability of the adaptive immune response against novel antigens is a robust measure of immunotoxicity, which supports that PFAS are able to suppress functional abilities of the developing immune system. A growing body of evidence concerning other developmental immunotoxicological outcomes, including responses such as allergy and asthma, supports that PFAS exposure also can hyperactivate the developing immune system. These multiple lines of evidence support that the developing immune system is a sensitive target of PFAS exposure and that the outcome may depend on critical windows of development during exposure and other aspects associated with the exposure scenario. The focus of this talk will be on immune developmental processes that appear to be sensitive windows for PFAS exposure, the putative mechanisms by which PFAS affect the developing immune system, and how developmental immunotoxicity data can be used as a benchmark for managing health risks of PFAS exposure.

S 1024 Derivation of Health-Protective Water Guidance for Bioaccumulative PFAS Chemicals Requires Incorporation of Placental and Breast Milk Exposure Pathways

H. Goeden. *Minnesota Department of Health, Minneapolis, MN.*

The standard approach for calculation of noncancer health-based water guidance uses a static equation involving the reference dose (RfD, mg/kg-d), a water intake rate (IR, L/kg-d), and relative source contribution (RSC) factor. PFOA, PFHxS, and PFOS, however, bioaccumulate in serum, cross the placenta, and are excreted into breast milk. As a result, chronic maternal exposures can have a large impact on nursing infant exposure. Although exposures during infancy are relatively brief, this life stage is of particular concern because (1) development is a toxicologically sensitive window; (2) infants consume a much greater volume of liquid per unit body weight than older children and adults; and (3) short-term exposures that occur during infancy can result in body burdens that take years to eliminate. The Minnesota Department of Health (MDH) developed a novel, one-compartment, Excel-based toxicokinetic model to simulate daily serum levels from birth through adulthood (attainment of steady-state conditions). The model included indirect exposures via placental and breast milk transfer as well as age-specific liquid intake rates. Two exposure scenarios were evaluated, each starting at birth with a preexisting body burden from placental transfer from a chronically exposed mother: (1) an infant exclusively formula-fed with contaminated water starting at birth, followed by a lifetime of drinking contaminated water; and (2) an infant exclusively breastfed for 12 months, by a mother who continues to drink contaminated water, followed by a lifetime of drinking contaminated water. As seen generally in previous biomonitoring studies, modeling results confirmed breastfed infants accumulate much higher serum levels. Predicted peak serum levels exceeded steady-state levels by up to sixfold, with levels exceeding steady state for over ten years. To ensure protection of the most highly exposed and vulnerable segments of the population, the latest guidance values by the MDH for PFOA, PFOS, and PFHxS incorporated placental and breast milk exposure pathways.

S 1025 The European Food Safety Authority Assessment of PFAS in Food: Preliminary Results and Ongoing Work

T. Haldorsson. *University of Iceland, Reykjavik, Iceland.* Sponsor: J. Conley

In 2018, the European Food Safety Authority (EFSA) published a new risk assessment on PFOS and PFOA in food. After reviewing existing evidence, new tolerable weekly intakes (TWIs) of 13 and 6 ng/kg body weight per day were established for PFOS and PFOA, respectively, based on human studies. These TWIs were 100-1000 fold lower compared with a previous assessment by EFSA from 2008. With respect to early-life exposures, associations with reduced birth weight (for PFOS and PFOA) and reduced antibody response following vaccination (for PFOS and possibly PFOA) were considered critical and likely to be causal. Based on studies in adults, increase in total (and LDL) serum cholesterol (for both PFOS and PFOA) and serum alanine aminotransferase for PFOA was also considered critical and likely to be causal. Increase in serum cholesterol was used as a basis for deriving TWIs. Benchmark dose modeling showed that the derived TWIs were protective for the other three outcomes. Following publication of the opinion, several European agencies that had derived their own health-based guidance values based on animal data raised concerns and identified specific uncertainties in the EFSA opinion. Parallel to this process EFSA published a new guidance document on how to assess combined exposure to multiple chemicals. To address concerns raised and to explore the possibilities of assessing dietary exposures to PFAS as mixture, the TWIs established for PFOS and PFOA were published as provisional. The outcome of this ongoing work, including publication of a risk assessment for PFAS other than PFOS and PFOA, is expected by the end of the year 2019. In this presentation, the scientific basis behind use by EFSA of human studies for deriving TWIs for PFOS and PFOA will be explained. In addition, possibilities, pros, and cons of assessing PFAS as a combined group of chemicals will be reviewed briefly.

S 1026 Lysosome/Autophagy Dysregulation in Toxicity and Disease

S. Lu. *Pfizer Inc., San Diego, CA.*

Lysosomes, first discovered by Dr. Christian de Duve more than five decades ago, are acidic membrane-enclosed organelles filled with hydrolytic enzymes that digest macromolecules from the endocytic, autophagic, and phagocytic membrane-trafficking pathways. Over the past decade, several areas of investigation have blossomed and brought lysosomes back into the forefront

of cell biology. One such area is autophagy, which is now recognized as an essential process for maintenance of organelle integrity, catabolism of lipid droplets, and response to stress. Autophagy dysregulation, including lysosomal dysfunction, has been linked to aging and age-related diseases, including disorders of lipid and glucose metabolism, as well as cardiovascular and neurodegenerative diseases. One exciting recent development is the recognition that lysosomes are the key regulators of signaling processes that regulate metabolism. The key metabolic signaling regulator mechanistic target of rapamycin (mTOR), for instance, needs to be recruited to the lysosomal surface to become activated and to sense cellular nutritional status. In addition, we now have a much better understanding of the mechanisms underlying the regulation of lysosome formation. The transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, drives expression of both autophagy and lysosomal genes. Many compounds can impact autophagic function either by lysosomal accumulation (e.g., tamoxifen and amiodarone) or inhibition of certain machinery (e.g., arsenic and tetanus neurotoxin) required for autophagy flux and fusion with the lysosome. The impact of lysosomal dysfunction by these compounds could lead to toxicity manifestation via several different mechanisms: (1) cell death associated with lysosomal membrane permeabilization; (2) membrane trafficking perturbation, including phagocytosis and autophagy; and (3) signaling modulation, including mTORC1 and Nrf2. In this session, we will focus on the discussion of adaptive changes associated with lysosomal stress, cell death by lysosomal membrane permeabilization, and Nrf2 signaling modulation associated with lysosomal/autophagy impairment. Examples will be presented that showcase the relationship between lysosomal/autophagy impairment and liver injury or cancer. The discussion highlights lysosome dysfunction as an essential mechanism for xenobiotic-induced toxicity, which could have a vast impact on the drug discovery process by providing an alternative means for organ toxicity mediated by physicochemical properties and lysosomal function perturbation besides on-target and off-target effect.

S 1027 Lysosomal Adaptation: How Cells Respond to Lysosomotropic Compounds

S. Lu. *Pfizer Inc., San Diego, CA.*

Lysosomes are acidic organelles essential for degradation and cellular homoeostasis, and recently, lysosomes have been shown as a signaling hub to respond to the intra- and extracellular changes (e.g., amino acid availability). Compounds including pharmaceutical drugs that are basic and lipophilic will become sequestered inside lysosomes (lysosomotropic). Following compound accumulation in the lysosome, many detrimental events can occur, including cell death and the perturbation of autophagy and phagocytosis, which could serve as a potential contributing factor in drug-induced organ toxicity. Lysosomal dysfunction by lysosomotropic compounds also can lead to compensatory responses, including nuclear translocation of transcriptional factors TFEB, TFE3, and MITF. The adaptive changes are protective to the cells under lysosomal stress. Mechanistic studies implicate calcium and mTORC1 modulation involvement in the adaptive changes. These results indicate that lysosomotropic compounds could evoke a compensatory lysosomal biogenic response but with the ultimate consequence of lysosomal functional impairment. This work also highlights a pathway of response to lysosomal stress and evidences the role of TFEB, TFE3, and MITF in the stress response.

S 1028 The Cross Talk of Lysosome, Autophagy, and NRF2

D. Zhang. *University of Arizona, Tucson, AZ.*

The autophagy-lysosome pathway is a key intracellular degradation pathway that plays an integral role in maintaining cell survival under stress conditions. As such, dysregulation of the autophagy pathway is associated with a variety of diseases. Importantly, autophagic dysfunction results in the accumulation of intracellular components, resulting in deleterious effects on the proteostatic, metabolic, and redox balance of the cell. One key protein implicated in autophagy-dysfunction-driven diseases is the cargo adaptor p62/SQSTM1, whose accumulation results in the aggregation of its binding partners and ubiquitinated targets into protein-rich inclusion bodies. Among the proteins sequestered by p62 is kelch-like ECH associated protein 1 (KEAP1), a key negative regulator of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2). Our lab and others have shown that p62-dependent activation of NRF2 plays an important role in the pathogenesis of numerous disease states, particularly those associated with chronic exposure to the environmental toxicant arsenic, including lung cancer and type 2 diabetes. Critically, arsenic-induced autophagic dysfunction occurs in the later stages of the autophagy pathway, as arsenic exposure prevents fusion of the autophagosome with the lysosome by disrupting formation of the STX17-SNAP29-VAMP8 SNARE complex. Arsenic inhibits SNARE complex formation via enhanced O-GlcNAcylation of SNAP29, with knockdown of OGlcNAc transferase (OGT), the enzyme that adds UDP-GlcNAc residues to proteins, or transfection of O-GlcNAcylation-defective, but not wild-type, SNAP29 into SNAP29 knockout cells preventing arsenic-mediated autophagy inhibition. Further studies also indicate that arsenic inhibits the retrograde and anterograde movement of autophagosomes, endosomes, and lysosomes, indicating the pleiotropic effects that arsenic has on not only autophagic function, but intracellular trafficking as well. Finally, our studies also support the *in vivo* relevance of p62-dependent activation of autophagy and NRF2 in ameliorating a liver fibrosis model. Therefore, NRF2 activation through autophagy upregulation is disease protective, whereas autophagy blockage is promoting arsenic-associated lung carcinogenesis and type 2 diabetes. Our studies indicate the importance of the context-dependent NRF2 activation in human disease.

S 1029 TFEB-Mediated Lysosomal Biogenesis Protects against Alcohol and Acetaminophen-Induced Liver Injury in Mice

W. Ding. *University of Kansas Medical Center, Kansas City, KS.*

Hepatic homeostasis is tightly regulated by autophagy and lysosomal degradation in the liver. We previously demonstrated that activation of autophagy protects against alcohol- and APAP-induced liver injury. Lysosomes sit at the last step of autophagy, which is critical for completing the autophagic process. Lysosomal biogenesis is regulated by a master transcription factor EB (TFEB). In this presentation, we will discuss the recent findings of how alcohol and APAP impair hepatic TFEB function, resulting in decreased lysosomal biogenesis and insufficient autophagy in hepatocytes. Impaired TFEB-mediated lysosomal biogenesis leads to accumulation of damaged mitochondria and excess lipid droplets, which exacerbates alcohol-induced steatohepatitis. Our unpublished data also show that liver-specific TFEB KO mice exacerbated whereas overexpression of TFEB in mouse livers protected against APAP-induced liver injury. Mechanistically, we found that liver-specific TFEB KO mice had high levels of APAP adducts and increased c-Jun N-terminal kinase (JNK) activation. In contrast, overexpression of TFEB increased lysosomal biogenesis and decreased levels of APAP adducts and JNK activation, likely via autophagic removal of APAP adducts. In conclusion, our findings indicate that activation of hepatic TFEB increases biogenesis of both lysosomes and mitochondria that protects against alcohol- and APAP-induced liver injury by removing damaged mitochondria and improving liver repair in mice.

S 1030 Interplay of Mitochondria, Mitophagy, and Lysosomes in Pathophysiology

J. J. Lemasters. *University of South Carolina, Charleston, SC.*

Mitophagy (mitochondrial autophagy) is an essential quality control mechanism that removes for lysosomal digestion damaged, effete, and superfluous mitochondria. In hepatocytes, nutrient deprivation and cellular remodeling initiate mitophagy of normally polarized mitochondria by a phosphoinositide-3-kinase (PI3K)/beclin1-dependent pathway, whereas mitochondrial damage initiates mitophagy by PI3K-independent pathways. Photodamage to individual mitochondria leads to swelling, rupture of outer membranes, and herniation of the inner membrane into the cytosol. Microtubule-associated protein 1A/1B-light chain 3 (LC3) and p62/sequestosome-1 then associate with the bare inner membrane, leading to autophagosomal sequestration and processing into lysosomes. Onset of the mitochondrial permeability transition (MPT) also causes mitochondrial swelling and rupture, which leads to clearance by mitophagy when limited numbers of mitochondria are damaged and to apoptosis and/or necrotic cell death with greater numbers. Conversely, lysosomal disruption can lead to mitochondrial damage and dysfunction. Lysosomal alkalization by inhibition of the vacuolar proton-pumping ATPase by ATP depletion or bafilomycin and lysosomal membrane disruption during acetaminophen hepatotoxicity causes lysosomal release of cheatable iron and uptake of Fe2+ into mitochondria via the mitochondrial calcium uniporter. Inside mitochondria, Fe2+ catalyzes hydroxyl radical formation, oxidative stress, MPT onset, and cell death. In response to acute ethanol feeding, widespread, reversible hepatocellular mitochondrial depolarization occurs to facilitate ethanol metabolism, but that activates mitophagy. Chronically, processing of depolarized mitochondria by mitophagy becomes compromised, leading to release of mitochondrial damage-associated molecular pattern molecules (mtDAMPs) that promote inflammatory and profibrogenic responses associated with alcoholic liver disease. Overall, the interplay between mitochondria, mitophagy, and lysosomes contributes importantly to pathophysiology.

1031 Control of Lysosomal Membrane Integrity: Opportunities for Clinical Intervention

M. Jäättelä. *Danish Cancer Society Research Center, Copenhagen, Denmark.* Sponsor: S. Lu

Lysosomes, with their over 50 hydrolytic enzymes, degrade old organelles and macromolecules for recycling. Leakage of lysosomal hydrolases into the cytosol causes so-called lysosome-dependent cell death, which provides an alternative for cells' normal suicide program apoptosis. In cancer cells, normally cytosolic chaperone, heat shock protein 70 (Hsp70), finds its way into the lysosomes, where it enhances the activity of several lipid hydrolases, thereby stabilizing the lysosomal membrane. Thus, cancer cells can avoid cell death by sending one of their essential proteins to work in a new location. These data have prompted us to develop strategies to inhibit the function of lysosomal Hsp70 by so-called cationic amphiphilic drugs (e.g., antihistamines and antidepressants) to treat cancer and to increase lysosomal Hsp70 activity to treat lysosomal storage disorders and other degenerative diseases. Here, our recent data that have led to clinical trials testing these strategies in patients and identification of signaling pathways that control lysosomal membrane integrity will be presented.

1032 New Data and Tools for Understanding Chemical Distribution *In Vitro*

N. Kramer. *Universiteit Utrecht, Utrecht, Netherlands.*

This session will explore how new experimentally evaluated models for *in vitro* chemical distribution are needed to better implement the US National Academy of Sciences *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Tox21, EU-ToxRisk, and the TransQST are exemplary projects where *in vitro* toxicity testing systems are being adapted to assess the toxicity of compounds, from pharmaceuticals to industrial chemicals. A critical research need for *in vitro* testing is to distinguish between the nominal concentration of the test chemical applied to the assay system and the effective concentration of the chemical at the site of action *in vitro*. Since chemicals are expected to vary in their distribution, it is essential that this is accounted for in determining relative potency in *in vitro* assays, and *in trans*lating *in vitro* findings to *in vivo*. While mathematical models exist to make such predictions, actual experimental data allowing evaluation have historically been scarce. The presenters in this Symposium are collecting new datasets and establishing novel methods to address *in vitro* distribution of chemicals. The session will begin with a presentation on new mathematical models based on physicochemistry to predict *in vitro* disposition. The next three presentations will describe new experiments measuring disposition *in vitro*, with a focus on model evaluation and interpretation of results in the context of high-throughput screening (HTS). These efforts include a major effort by the Tox21 collaboration to evaluate the impact of these issues across a wide chemical space, including both pharmaceuticals as well as other chemicals occurring in the environment and commerce. The session will conclude with a discussion of lessons learned and challenges remaining with respect to *in vitro* chemical distribution and translation to *in vivo* from the EU-ToxRisk and TransQST consortiums. The session will consider (1) What data are available today for evaluating differences between *in vitro* nominal and effective concentrations? (2) What approaches exist for extrapolating from these data to untested and novel chemical structures? (3) What experimental methods are available for measuring *in vitro* disposition? and (4) What is the impact of *in vitro* disposition on chemical risk prioritization?

1033 A Comparison of Kinetic Model Predictions and Analytically Determined Distribution of Test Chemicals in 2D, Sandwich and 3D, Spheroid Hepatocyte Models

N. Kramer. *Universiteit Utrecht, Utrecht, Netherlands.*

The absorption, distribution, metabolism, and excretion (ADME) of chemicals play a central role in quantitative *in vitro in vivo* extrapolation (QIVIVE) studies, as these processes determine the concentration of the chemical at the target organ where the toxic effect is initiated *in vivo*. Although largely ignored, similar kinetic processes determine the target concentration and thus the level of bioactivity of chemicals in *in vitro* assays. Despite chemicals or assays eliciting similar effects at similar nominal concentrations, the bioavailable concentration may vary greatly between chemicals and assays, thus hampering comparisons. A number of partition models have been developed to estimate target concentrations of a chemical *in vitro*. The aim of this study was to review these models in terms of their chemical and assay applicability domains, inclusion of kinetic processes, input parameters, and the extent to which they have been evaluated. Subsequently, model predictions of the distribution of 10 chemicals varying in physicochemical and toxicokinetic properties were compared with measured time-resolved concentrations of these chemicals associated to well plate plastic, exposure medium, cells, and cell attachment matrices in an intrinsic clearance assay with HepaRG cultured as a monolayer, sandwich, and spheroid. The free concentration varied significantly for the lipophilic, quickly cleared chemicals between models as a result of the variation in phases and kinetic processes included by the models. The virtual cell based assay (VCBA) model resulted in distribution-time profile of the chemicals that corresponded with analytical measurements.

1034 Biokinetic Modeling of *In Vitro* Systems for Toxicity Testing and Risk Assessment

C. Fisher. *Certara UK Limited, Sheffield, United Kingdom.*

The vision for toxicity testing and risk assessment in the 21st century aims to increase the use of human-relevant *in vitro* model systems and reduce, refine, and ultimately replace the use of animal models. An important component of successfully implementing this strategy is the use of modeling and simulation to translate the toxic effects and associated concentrations at which these effects are observed from the *in vitro* to *in vivo* situation. Biokinetic modeling allows prediction of the concentrations of chemicals within the cells of *in vitro* test systems. Such mathematical models also allow the assessment of the metabolism, transport, and dissolution/solubility of drugs and other chemicals. The use of such modeling approaches facilitates the parameterization of physiologically based toxicokinetic (PBTK) models enabling the translation of the intracellular concentrations, exerting effects in *in vitro* systems, to predicted concentrations in the tissues under various exposure scenarios. Drawing on experiences from within the EU-ToxRisk program and the TransQST (Translational Quantitative Systems Toxicology) consortium, as well as the wider literature, the advantages and current challenges in using biokinetic and PBTK models as part of an integrated approach to chemical testing and risk assessment will be discussed.

1035 Tox21 Partnership on *In Vitro* Dispositions: Initial Results

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Understanding the biokinetics of chemicals evaluated in alternative models is an important step in extrapolating these results to humans. In an effort to extrapolate *in vitro* media concentrations to *in vivo* exposures (IVIVE), pharmacokinetic models are used to estimate human oral exposures that would result in blood concentrations equivalent to the nominal media concentrations. One of the uncertainties of this assumption is that the ratio of the chemical concentration in media to cells is equivalent to the ratio of chemical concentration in blood to tissue. Another uncertainty of this assumption is the static nature of the cell culture exposure compared with the kinetic processes occurring *in vivo*. In Tox21, most of the assays employ transformed cell lines cultured in monolayers in plastic wells. These cells have limited metabolic capabilities and the chemical can distribute between the plastic, media, and cells. In contrast, human exposure is a dynamic process in which absorption, distribution, metabolism, and elimination influence blood and tissue chemical concentrations. These initial studies evaluate the *in vitro* distribution of chemicals over 24 hours, the length of many of the Tox21/ToxCast assays. The development of a dataset that quantifies the concentration of chemicals in the media, plastic, and cells provides insight into the magnitude and directionality of the uncertainty in the assumption that nominal media concentrations and blood concentrations are equivalent. To date, MCF-7 cells have been exposed to a diverse set of 10 chemicals. Results from this initial study show that at 24 hours the concentration of chemical in cells compared with the concentration in media can vary from threefold (atrazine) to 128-fold (triphenyl phosphate). Comparisons were made between experimental cellular concentrations and predicted cellular concentrations using the model by Armitage and coworkers. The comparisons showed decent agreement with a log RMSE of 0.88. The Armitage model appears to account for some of the trends observed in our experimental data.

1036 The Influence of *In Vitro* Disposition and Toxicokinetics on the Association of *In Vitro* Bioactivity and *In Vivo* Toxicity Data

G. Honda. *US EPA/NCCT, Research Triangle Park, NC.* Sponsor: J. Wambaugh

This presentation will provide clear, statistical evidence that *in vitro* disposition modeling and physiologically based toxicokinetics (PBTK) enable improved association of *in vitro* bioactivity and *in vivo* toxicity data via *in vitro* to *in vivo* extrapolation (IVIVE). To use high-throughput screening (HTS) assays as an alternative to traditional animal studies, we must link *in vitro* bioactivity concentrations and toxic doses by IVIVE. Previously, it has not been clear whether the use of IVIVE even improves the observed association between *in vitro* bioactivity and *in vivo* toxicity data. Further, there is currently no single, optimal approach to IVIVE. Generally, toxicokinetic models may be used to determine chemical concentrations (e.g., unbound plasma, total plasma, and tissues) that correspond to a dose where a toxic effect was observed *in vivo*, while *in vitro* disposition models may be used to describe the concentrations (i.e., free and cellular) that correspond to a nominal concentration where bioactivity was observed in an HTS assay. We have used an *in vitro* disposition model and a high-throughput, PBTK model to relate *in vitro* bioactivity (ToxCast) and endpoint-specific rat *in vivo* toxicity data. For every possible comparison of *in vitro* and *in vivo* endpoint, the concordance between the *in vivo* and *in vitro* data was evaluated by a regression analysis. We tested various sets of IVIVE assumptions and demonstrate that the combination of PBTK and *in vitro* disposition modeling improves our ability to observe the association between *in vitro* bioactivity and *in vivo* toxicity data. Potency values from *in vitro* screening should therefore be transformed by IVIVE to build better machine-learning and other statistical models for predicting *in vivo* toxicity in humans. *This abstract does not necessarily reflect US EPA policy.*

1037 Applicability Domains and Future of Nonanimal Tests for Skin Sensitization

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Allergic contact dermatitis is an undesired side effect observed with many products, including cosmetics, natural extracts, drugs, chemicals, and medical devices. Over the last decades, a great deal of progress has been made in the development of alternative *in vitro* testing strategies to assess these issues, concurrent with the mechanistic understanding provided by the adverse outcome pathway (AOP) framework. The use of animals in toxicology is under ever-increasing scrutiny, with mounting pressure to develop effective alternatives. Efforts should be devoted to developing reliable *in vitro* assays and integrated testing strategies capable of addressing toxicity concerns for a broad spectrum of products and chemicals. This will require a better understanding of the applicability domains of scientifically validated assays and methods that are currently being used so that chemicals can be tested appropriately in these assays to produce valid predictions. In addition, accurate definition of the applicability domains will facilitate modification and improvement of current and new assays to expand these domains, resulting in better coverage of the chemical space for prediction of sensitization potential. The purpose of this Workshop is to cover current knowledge on the applicability domains of these methods and to understand their limitations and the opportunities they offer. The session will open with a brief introduction by the session Chair, followed by four presentations aimed at defining the current state of the applicability domains for *in vitro* approaches as well as recent progress to expand these domains. The first presentation will present the current status of an international collaboration charged with establishing international test guidelines for nonanimal testing strategies that would serve as full replacements to the animal tests for skin sensitization. The approach includes using the current nonanimal methods within their applicability domains to model skin sensitization. The second presentation will define the applicability domains and limitation for the individual OECD Test Guidelines 442C, 442D, and 442E, which address chemical peptide reactivity, keratinocyte activation, and dendritic cell activation, respectively. A complete understanding of the influence of chemistry on these factors is critical for accurate predictions and improvement of current approaches to expand the applicable chemical space that can be tested without the use of animals. The third presentation will focus on *in chemico* assessment of peptide reactivity, presenting a characterization of the currently validated Direct Peptide Reactivity Assay (DPRA) approach while providing insight into the novel Peroxidase Peptide Reactivity Assay (PPRA), which holds promise to expand the applicability domain of peptide reactivity assays to include pre- and pro-haptens requiring metabolism. The final presentation will provide initial data for a novel dendritic cell activation assay, the THP-1 Activation Assay, which is being developed to expand the applicability domain of the current *in vitro* dendritic cell activation assays to include drugs that exhibit limited or no cytotoxicity. Overall, this Workshop aims to better define the applicability domains for nonanimal sensitization testing and identify progress and opportunities for expanding these domains. This Workshop represents an international collaboration between the Immunotoxicology Specialty Section of SOT and the Immunotoxicology and Chemical Allergy Specialty Section of EUROTOX in an effort to communicate and improve the science of alternative approaches for assessing potential for skin sensitization.

1038 Establishing International Test Guidelines for Skin Sensitization: Predictive Capacity and Applicability Domain of Nonanimal-Defined Approaches

N. Kleinstreuer. *NIEHS/NICEATM, Morrisville, NC.*

Following the work done by international consortiums such as Cosmetics Europe and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), a project to develop a Test Guideline on Defined Approaches (DAs) for Skin Sensitization, co-led by the US, the EU, and Canada, was officially included on the Organisation for Economic Co-operation and Development (OECD) workplan in April 2017. This work aims to establish Test Guidelines for nonanimal testing strategies (defined approaches) that fall under the Mutual Acceptance of Data (MAD) clause. MAD means that any of the 36 Member countries will accept data generated under OECD Test Guidelines, avoiding redundant testing and ensuring international harmonization. This proposal is the first attempt to establish international test guidelines for nonanimal testing strategies that would serve as full replacements to the animal test for skin sensitization, a regulatory requirement across many jurisdictions and chemical sectors. The OECD national coordinators agreed in April 2018 on a final evaluation framework to be applied to DAs for hazard identification, potency categorization, and eventually quantitative risk assessment, and an expert group was formed to apply this framework to several nonanimal DAs. A subsection of the expert group is specifically examining the applicability domain of DAs, and how it is affected by the individual test elements, both *in vitro* and *in silico* in nature. Additional subgroups are focused on curating reference datasets, in particular human evidence, and characterizing uncertainty for the *in vitro* test components, the DAs, and the *in vivo* data. Based on the progress made in this area, the US Environmental Protection Agency released a draft science policy in 2018 to accept two nonanimal DAs as replacements for the mouse test, for pesticide active ingredients, inerts, and other single-constituent chemicals, and other regulatory authorities are considering similar actions. Work is ongoing to target unresolved challenges for risk assessment, such as potency estimation and assessing DA performance on mixtures. Overall, this project represents a major milestone toward the replacement of animal testing for the endpoint of skin sensitization, both within and outside the US.

1039 Chemistry behind Sensitization: Possible Limitation and Bottleneck of *In Vitro* Methods

R. Landsiedel. *BASF SE, Ludwigshafen, Germany.*

Three *in vitro* methods have gained regulatory acceptance for the prediction of skin sensitization (OECD Test Guidelines 442C, -D, -E). The OECD Test Guideline 442C addresses the molecular initiating event on the adverse outcome pathway (AOP) for skin sensitisation of covalent binding of chemical sensitizers to skin proteins and details the *in chemico* method DPRA. The OECD Test Guideline 442C addresses the second key event in the AOP for skin sensitization, that of keratinocyte activation with the ARE-Nrf2 pathway being central to the response. The guideline details two *in vitro* methods using ARE-Nrf2 luciferase constructs to signal changes in keratinocytes induced by sensitizers, the Keratinosens and Lusens assays. The OECD Test Guideline 442C addresses the key event on the AOP for skin sensitization of dendritic cell activation and details two *in vitro* methods for testing this event, the Human Cell Line Activation Test (hCLAT), the U937 Cell Line Activation Test (U-SENS), and the Interleukin-8 Reported Gene Assay (IL-8 Luc). The applicability domains and limitations of these *in chemico* and *in vitro* methods need to be described to assess their appropriateness for the test substance and the reliability of the predictions. This is a critical aspect that must be considered for industrial users and regulatory agencies. To answer this question, BASF, in collaboration with other academic partners and industrial associations, has evaluated and compared the different methods for their predictive capacities. The purpose of this presentation is to present and discuss the applicability domains for these assays and possible limitation of *in vitro* methods, including the precision of the methods and the predictivities according to the reaction mechanism of the hapten.

1040 The Use of Peptide Reactivity Assays for Skin Sensitization Hazard Identification and Risk Assessment

C. Ryan. *Procter & Gamble, Mason, OH.*

Over the past 20 years or more, investigators have been developing non-animal test methods for use in assessing the skin sensitization potential of chemicals. In parallel with this effort, the key biological events of skin sensitization have been well characterized in an AOP proposed by the OECD. The key molecular initiating event of this AOP is haptenation or covalent modification of epidermal proteins. In this review, the strengths and limitations of the DPRA are described in the context of the applicability domain for this *in chemico* technique. The DPRA has been formally validated and incorporated into an OECD Test Guideline (TG442C). The DPRA shows promise for assisting in hazard identification as well as for assessing skin sensitization potency when used in an integrated testing strategy. Research has shown that chemicals requiring metabolism often fail to produce appropriate results in the DPRA. These pro- and pre-haptens are outside the applicability domain of the DPRA, and predictions are questionable. Recent advances in the *in chemico* space resulted in the development of the PPRA. This assay uses peroxide enzyme systems to provide the metabolic activation of pre- and pro-haptens, resulting in increased predictive value of the peptide reactivity value. The PPRA provides a valuable opportunity to expand the applicability domain for *in chemico* assessment of the key molecular initiating event. This talk will discuss the methodology, applicability, and limitations associated with peptide reactivity assays as valuable tools in the identification of skin sensitizers.

1041 Drug-Induced Hypersensitivity: *In Vitro* Opportunities

E. Corsini. *Università degli Studi di Milano, Milan, Italy.*

Starting from the assumption that allergenic drugs share common mechanisms of cell activation with chemical allergens, it is possible to speculate for drugs, as it has been done for chemicals, that human drug allergens can be predicted through identification of peptide-bound reactive small chemical drugs to drug-specific naive human T cell priming. According to published literature, it also may be possible based on careful selection of molecular markers (e.g., cytokines, surface markers, microRNA) to discriminate between different types of hypersensitivity induced by drugs. For example, the intrinsic capacities of allergens to polarize conventional dendritic cells (cDC) toward type 1 cDC or type 2 cDC, irrespective of local factors such as those determined by cutaneous or mucosal epithelial microenvironments, is a key factor. In currently validated *in vitro* methods (hCLAT, U-SENS, and IL-8 Luc), addressing key event 3 (DC activation), very few drugs have been tested, and all were correctly classified. To extend on the current methods, we developed a method based on CD54/CD86 upregulation in THP-1 together with assessment of IL-8 production to identify allergenic drugs, with encouraging results. The addition of the assessment of IL-8 production proposes to expand the applicability domain for *in vitro* assays addressing the key event of DC activation, as DC production of IL-8 is known to result in chemotaxis of other cellular mediators of inflammation and allergy—namely, neutrophils and T cells—thereby covering both antigen processing and presentation and cell recruitment functions of DCs. Initial testing of the method with known allergenic drugs has demonstrated that the assay correctly classifies these drugs as sensitizers even when metabolic activation is required. Importantly, this assay expands to applicability domain of *in vitro* methods for interrogation of DC activation to chemicals and drugs that show limited or no cytotoxicity, as these chemicals are not well predicted by the hCLAT assay. While without any doubt additional efforts and extensive resources are necessary to improve preclinical testing methodologies, including optimization of the experimental design and interpretation of data, the possibility of using currently *in vitro* methods for the hazard identification of the allergenic potential of drugs will be presented and discussed.

1042 Known Unknowns: Challenges and Approaches for Handling Chemical, Hazard, and Regulatory Uncertainty in Medical Device Safety Assessments

T. Lewandowski. *Gradient, Seattle, WA.*

The regulatory landscape for the safety evaluation, clinical testing, and commercial development of medical devices is undergoing considerable changes, including new requirements for material characterization and chemical risk assessment early on in the development process. In this dynamic environment, extractables/leachables (E/L) analysis is becoming a key tool in biocompatibility assessments to ensure patient safety and establish regulatory compliance. The first speaker will begin the discussion on medical device chemical characterization strategies, a necessary step for understanding potential chemical exposures from medical device components. The presentation will include examples of how information concerning material chemistry and the manufacturing process can reduce the cost and effort associated in resolving "unknown" extractable compounds. The next presentation will focus on predictive toxicology methods (e.g., computational toxicology programs, read-across, Threshold of Toxicological Concern) for evaluating potential risks from extractable compounds. Case studies will be presented to demonstrate the importance of expert judgment when interpreting *in silico* hazard predictions, as well as approaches for justifying a read-across approach for risk assessment of extractable compounds. The third speaker will then discuss the US Food and Drug Administration (US FDA) perspective on the issues raised in the preceeding talks. Agency experience with unique nontargeted analytical methods that generate data adequate for toxicological risk assessment will be presented, which include, but are not limited to, extraction method design, analytical instrument/tool selection, selecting an analytical evaluation threshold (AET), sample manipulation, system suitability, calibration, identification/semi-qualification, and data reporting. The final speaker will present a broader overview of the global regulatory landscape for medical device safety evaluation. Notable activity includes the revision of ISO 10993-1, implementation of the European Union's revised Medical Devices Regulation, and amendments to California Proposition 65. This presentation will cover how new requirements for extractables/leachables analysis will affect the manufacturer's ability to justify the safety of hazardous substances within devices, verify warning label exemption, evaluate biological equivalence of predicate/proposed devices, and support supply chain controls and ensure efficient change management.

1043 Chemical Characterization Strategies for Medical Device Biocompatibility Assessment

A. Kozak. *Cambridge Polymer Group Inc., Boston, MA.* Sponsor: T. Lewandowski

This presentation will discuss modern chemical characterization strategies for the toxicological risk assessment of medical devices. Following the ISO 10993 framework, the process of evaluating medical device materials of construction, manufacturing processes (including cleaning and sterilization), and end use environment to justify selection of extraction conditions and analytical techniques will be discussed. As will be presented, a robust medical device chemical risk assessment workflow considers the device materials of construction and identifies potentially extractable species before testing. This includes reviewing polymer resins and metal alloys, potential additive/impurity profiles, sterilization agents, and manufacturing processing aides such as detergents, fiber spin finishes, and machining oils (whose composition is often proprietary). Such considerations of the product chemistry not only ensure that the analytical techniques selected are well suited to the detection of suspected extractables, but also facilitate identification of compounds and mitigate the cost and effort associated in resolving "unknown" peaks that may not be present in conventional mass spectral libraries. This presentation also will discuss how consideration of device morphology, surface area, and end use conditions also are integral in selecting justifiable extraction conditions. Strategies for sample extract "workup" will be reviewed, with special considerations for mitigating volatile loss while ensuring adequate sensitivity for trace chemical detection. Specific case studies will include dealing with challenging materials of construction, highly porous morphologies, and identification of polymeric sterilization by-products. By considering chemical risk in tandem with the medical device development process, ways whereby medical device manufacturers can avoid surprises and alleviate delays to market or regulatory approval will be highlighted.

1044 Predictive Toxicology Approaches for Medical Device Biocompatibility Assessment

J. Cohen. *Gradient, Cambridge, MA.*

Comprehensive toxicological characterization of extractable/leachable substances is an essential component of a medical device biocompatibility assessment. Chemical analysis often identifies unique or complex extractable chemical structures that lack complete toxicological data packages. Best practices for using new approach methodology (NAM) predictive tools to evaluate these detected chemicals have yet to be formally established. This presentation will provide an overview of predictive toxicology tools currently available for evaluating risks to human health, along with a discussion of potential pitfalls and best practices. The ICH M7 Guideline provides a clear method for applying computational toxicology programs to predict mutagenic potential, which can then be applied to establish an appropriate Threshold of Toxicological Concern. However, such programs must not be used as a black

box. Predictions should be evaluated using expert judgment to support a risk conclusion based solely on computational methods. Furthermore, computational toxicology predictions for other hazard endpoints (e.g., developmental or reproductive effects) are less well studied but are actively under development. Case studies will be presented to demonstrate the role of expert judgment when interpreting *in silico* hazard predictions for mutagenicity and skin sensitization endpoints. Read-across approaches relying on experimental data for chemicals sharing similar structural, physical, and chemical properties, along with a similar anticipated mechanism of action, may provide additional information for establishing appropriate safety limits for extractable compounds lacking data. Additional case studies will present approaches for justifying read-across for risk assessment of extractable compounds lacking toxicity data.

1045 CDRH Scientific Perspective on Material Characterization and Toxicological Risk Assessment of Nontargeted Medical Device Extractables

B. Oktem. *US FDA/CDRH, Silver Spring, MD.*

Medical devices are unique in the diversity of materials of construction, extent/types of tissue contact, and duration of contact. When evaluating the safety of medical device extractables, it is these unique attributes that necessitate medical device-specific analytical/toxicological considerations, which will be presented. In absence of *a priori* knowledge of a material's complete chemical composition, an extractable study, nontargeted chemical analysis, and toxicological risk assessment are performed to determine whether expected or unexpected extractables (e.g., additives, degradants, or impurities) present in/on patient-contacting components of the medical device raise a toxicological concern. Nontargeted chemical analysis generally involves extracting the medical device in multiple solvents of diverse polarities, followed by use of multiple analytical instruments with appropriate sensitivity for identification and semi-quantification. Considerations of unique nontargeted analytical methods that generate data adequate for toxicological risk assessment will be presented, which include, but are not limited to, extraction method design, analytical instrument/tool selection, sample manipulation, system suitability, calibration, identification/semi-qualification, and data reporting. Case studies will demonstrate how risk assessment approaches and considerations may depend on the nature and use of different medical devices (e.g., permanent implant versus skin contact device versus indirect contact devices). The selection and application of the analytical evaluation threshold (AET) also will be discussed. Lastly, the US FDA perspective on the unique chemical/toxicological considerations that aid the analytical chemist and toxicological risk assessor in prioritizing nontargeted extractables for toxicological risk assessment also will be discussed.

1046 Extractables and Leachables Analysis of Medical Devices in a Changing Global Regulatory Environment

W. Christian. *Medtronic, Jacksonville, FL.*

The medical device industry is currently experiencing changes in the regulatory landscape with respect to biocompatibility and hazardous substances requirements, which are aimed at improved patient safety assurance for devices on the market. Recent events in which patient safety was compromised by loose regulations and testing requirements have motivated much of the present-day regulatory reform. Notable activity includes the revision of ISO 10993-1, entry into force of the European Union Medical Devices Regulation, and amendments to California Proposition 65. A common theme among the regulatory reform is the addition of requirements to understand the chemical content of medical devices and, in turn, the toxicological consequences associated with patient exposure to that chemical content. Therefore, in this dynamic regulatory environment, extractables/leachables analysis is becoming a key tool in establishing compliance, though its utility is applicable in more ways than one. An overview of the recent regulatory changes and examples of how extractables/leachables analysis can be utilized to assess patient/user exposure, justify the safety of hazardous substances within devices, verify warning label exemption, evaluate biological equivalence of predicate/proposed devices and also support supply chain controls, ensure efficient change management, and segue into modern *in silico* approaches to evaluating biocompatibility will be presented.

1047 Oligodendrocytes/Schwann Cells: Major Targets in Neurotoxicity and Neurological Diseases

H. Hogberg. *Johns Hopkins University, Baltimore, MD.*

Besides neurons, glial cells represent major targets of neurotoxicants. However, cell-based methods to assess glial toxicity are clearly underrepresented, especially when it comes to oligodendrocytes and Schwann cells. Their role is to form the myelin sheet around axons, which is crucial for neuronal function and one of the key processes in brain development. Significance of myelination cannot be understated, and any damage to myelin results in debilitating diseases. Failure to form or maintain myelin can disrupt neuronal signal transmission or trigger degradation of axons, and a reduction in the velocity of action potentials can lead to physical or mental disability and induce severe neurological symptoms. Potential mechanisms of oligodendrocyte/Schwann cell toxicity are inflammation, thyroid hormone disruption, glutamate excitotoxicity, disruption of cholinergic signaling, and oxidative stress. Moreover, several human neurologic diseases have been associated with dysfunctional oligodendrocytes and myelin deficits, such as schizophrenia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), periventricular leukomalacia (PVL), and chemotherapy-induced peripheral neuropathy (CIPN). Unfortunately, there is a lack of effective treatments to treat these disorders, despite decades of research using animal models in drug discovery, and pharmaceutical companies are desperately seeking new human-relevant alternatives. This Workshop will describe *in vitro* assays and nonmammalian species to assess toxicity to oligodendrocytes/Schwann cells and myelination. Moreover, the current and future use of these approaches in toxicity testing and drug discovery will be discussed with national and international perspectives from both academia and industry. The speakers have been selected to present applications of novel assays for oligodendrocyte/Schwann cells/myelination toxicity that mimic various parts of the nervous system and different developmental windows of the nervous system. The introduction will address the significance of myelination for neuronal function and how failure to form myelin and demyelination can lead to adverse outcome and disease. In addition, it will give a brief background and present the agenda of the Workshop, including the overall goals, speaker lineup, and the intended outcome. Firstly, the *in vitro* assay of oligodendrocyte differentiation and maturation from human neural precursor cells (hNPCs) and its use for developmental neurotoxicity (DNT) will be described. The second speaker will present the use of three-dimensional (3D) neural models to assess chemicals' potential to induce demyelination in the more mature central nervous system (CNS). Furthermore, the assessment of de- and remyelination of the peripheral nervous system (PNS) will be addressed using a 3D Nerve-on-a-Chip platform. The next speaker will introduce the use of the nonmammalian species *Xenopus laevis* to study demyelination-remyelination and the quantitative evaluation of behavioral perturbation after chemical exposure. The final speaker will present data on remyelination both in murine *in vivo* and *in vitro* models and describe efforts to determine the significance of these findings to a human *in vitro* model and individuals with demyelinating conditions. The main goal of the Workshop is to shed light and discuss the current status of assays for oligodendrocyte/Schwann cell toxicity and the translation to human adverse outcomes and diseases. Speakers have been encouraged to discuss their data, critical to identify gaps, limitations, and relevance to humans with their approaches. Presentations will be followed by an interactive panel discussion with the session speakers where e.g. limitations, species differences and extrapolation to the *in vivo* situation will be deliberated.

1048 The Human Oligodendrocyte Maturation Assay as a Test for Assessing Thyroid Hormone Disruption *In Vitro*

E. Fritsche. *IUF—Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.*

Testing for developmental neurotoxicity (DNT) according to the Organisation for Economic Co-operation and Development/US Environmental Protection Agency guidelines is performed in rats. Current efforts are aiming at setting up a DNT *in vitro* battery for regulatory application that should predict DNT. One mode of action how compounds can interfere with brain development is endocrine disruption. Here, a substance interferes with the production, transport, metabolism, or target cell effects of a hormone. Because thyroid hormone (TH) is crucial for brain development, we established an assay that identifies compounds that interfere with human neural progenitor cell (NPC) or oligodendrocyte TH signaling based on TH target gene mRNA expression. We also characterized the human NPC system for expression of components of the TH pathway, like TH transporters, diodinases, TH receptors, and TH target genes. NPC possess a functional TH signaling machinery that is involved in oligodendrocyte maturation of developing NPC. This test might be used as a screening assay to identify compounds acting as TH disruptors on the

developing brain in a relative short time frame (i.e., five days *in vitro*). Added to the DNT *in vitro* testing battery, tests for endocrine disruption will increase the applicability domain of such a screening effort.

1049 Myelination as an Endpoint for (Developmental) Neurotoxicity in 3D *In Vitro* Models

H. T. Hogberg[1], M. Chesnut[1], S. Gul Akgun Olmez[1], A. Kleensang[1], D. Pamies[2], and L. Smirnova[1]. [1]*Johns Hopkins University, Baltimore, MD; and* [2]*University of Lausanne, Lausanne, Switzerland.*

There is lack of *in vitro* assays that can assess myelination, despite it being an important key process for neuronal function and neurodevelopment and a potential target for neurotoxicants. Lately, it has become evident that more complex *in vitro* cell models, such as 3D test systems, are essential to reproduce the architecture and function of the central nervous system (CNS). The 3D structure is especially crucial for the myelination, as one oligodendrocyte myelinates axons from multiple neurons. We have previously developed different 3D neural models of the CNS using gyratory shaking based on rat primary cells and human induced pluripotent stem cells (iPSC). These models have shown to be relevant for key cellular processes involved in neurodevelopment, including proliferation, differentiation, apoptosis, synaptogenesis, intracellular signaling, and network function. In addition, the models present unique features, as they have shown *de novo* myelination. Both models show increase in myelination over the time of differentiation assessed with quantitative RT-PCR and immunohistochemistry for different oligodendrocyte markers (e.g., MBP, mag, O1, and O4). In addition, 3D reconstruction of confocal z-stacks images and electron microscopy confirm the wrapping of the myelin around the axonal structures. We have exposed both models to compounds shown to induce de- and dysmyelination in animals (cuprizone, pesticides, and flame retardants) at concentrations from 0.1 to 10µM for 7-14 days. Assessment of the different oligodendrocyte markers with RT-PCR and immunohistochemistry after the exposure indicates reductions in myelin formation at noncytotoxic concentrations. These 3D models could therefore be useful tools to assess compounds that induce de- and dysmyelination and have the potential also to screen drugs for remyelination.

1050 A 3D Nerve-on-a-Chip Platform of the Peripheral Nervous System to Assess De- and Remyelination

L. Curley. *AxoSim, New Orleans, LA.* Sponsor: H. Hogberg

The use of engineered 3D organoids has seen tremendous growth as a preclinical drug screening tool because they are more biomimetic and physiologically relevant than 2D assays. When modeling complicated tissues such as the human nervous system, 3D engineered cultures provide marked advantages by recapitulating cell-cell interactions. However, a limited focus has been given to microphysiological systems that mimic peripheral nerves (PNs), even though peripheral neuropathy is implicated in numerous disease states and is a common and significant side effect of many therapies. We have devised a novel method for engineering *in vitro* human iPSC-derived and primary rat 3D nerves that support axon growth analogous to PN anatomy. This *in vitro* nerve can provide clinically relevant metrics such as nerve conduction velocity (NCV) and histological ultrastructure, which are recognized as the gold standard in evaluating peripheral neuropathy. Commonly used spheroid fabrication methods were optimized to create co-culture spheroids of human iPSC-derived neurons and primary human Schwann cells. Over four weeks, the nerves were grown in a 3D environment to remarkable lengths of 5 mm in a growth-directing dual-hydrogel scaffold. Population-level electrophysiological testing revealed the nerve conduction velocity in the biomimetic human nerve to be 0.13±0.02 m/s. Schwann cell migration, ensheathing, and myelination occurred in the co-culture spheroids as confirmed by S-100 immunostaining, and lamination seen in TEM micrographs. This microphysiological model of human peripheral nerve simulated the multifaceted physiology of peripheral nerves, such as long unidirectional axonal outgrowth, myelination, and nerve conduction, and thus can be used for modeling a variety of neuropathic conditions *in vitro*, including myelination and demyelination.

1051 A Simple and Reliable Model to Investigate Myelination, Conditional Demyelination, and Remyelination

B. Zalc. *Sorbonne Université, Paris, France.* Sponsor: H. Hogberg

We have generated a *Xenopus laevis* transgenic allowing conditional ablation of myelinating oligodendrocytes. In this MBP-GFP-NTR line the transgene, GFP reporter fused to *E. coli* nitroreductase (NTR) driven by upstream regulatory sequence of myelin basic protein, is specifically and selectively expressed in myelin forming oligodendrocytes. Since NTR converts the innocuous pro-drug metronidazole to a cytotoxin, addition of metronidazole into the aquarium water induces ablation of oligodendrocytes and demyelination. As tadpoles are transparent, demyelination can be monitored *in vivo* and quantified. For instance, in stage NF52-53 (i.e., 25 days post-fertilization) MBP-GFP-NTR transgenic tadpoles treated for 10 days with metronidazole (10 mM), the number of GFP+ cells per optic nerve significantly decreased from 13.9 + 1.0 to 2.3 + 2 ($p<0.01$). Upon cessation of metronidazole treatment (i.e., after returning tadpoles to normal water) spontaneous remyelination occurred rapidly: the number of GFP+ oligodendrocytes per optic nerve reached 8.3 + 0.9 and 13.3 + 1.5 at 3 and 8 days of recovery, respectively. We confirmed that counting the number of GFP+ cells is a reliable indicator of the extent of myelination/demyelination/remyelination by immunolabeling with myelin-specific antibodies and electron microscopy. Furthermore, we reasoned that demyelination should translate into loss of sensorimotor functions. To challenge this hypothesis, we measured the speed and distance traveled before and after demyelination and during spontaneous remyelination. In addition, to test the functional consequence of demyelination and repair of the optic tract of our transgenic tadpole, we adapted a visual avoidance paradigm, based on a virtual collision test. Quantitative evaluation of behavioral perturbation was confronted to the degree of demyelination-remyelination assayed by counting the number of GFP+ oligodendrocytes in the optic nerve.

1052 Discovery of New Therapeutic Targets for Myelin Regeneration Using Murine and Human Models

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Myelin dysfunction is increasingly recognized as a prominent feature of diverse neurological conditions affecting the adult and developing central nervous system (CNS). Oligodendrocytes not only generate myelin in the brain and spinal cord thereby enhancing nerve impulse conduction, but also provide trophic and metabolic support to axons. Oligodendroglia are vulnerable to injury in multiple sclerosis and other neuropathologies, leading to conduction block, axonopathy, and permanent neurologic impairment. Although the CNS has an innate capacity for myelin regeneration, this is often incomplete, leaving axons vulnerable to degeneration. We recently discovered that the thrombin receptor, also known as Protease Activated Receptor 1 (PAR1), is rate-limiting in myelin development. Here, we will describe efforts to determine the significance of these findings to individuals with demyelinating conditions using a human 3D BrainSphere model that contains induced pluripotent stem cell (iPSC)-derived neurons, astrocytes, and myelinating oligodendroglia. In addition, we will highlight our efforts to determine if the robust promyelinating effects of blocking PAR1 function developmentally extend to myelin regeneration across both experimental murine *in vivo* and *in vitro* models, with complementary investigation of demyelination and remyelination in the human BrainSphere model. Together, results highlight the urgent need and value of utilizing experimental murine systems that harness the power of genetics in conjunction with human iPSC-derived 3D brain cultures that represent a high-throughput model for screening drug candidates and that represent the first tangible steps toward clinical translation.

1053 Toxicological Exposure and Risk Assessment of Emissions from 3D Printers

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3D printing, also referred to as additive manufacturing, is a rapidly growing technology that is expected to have a significant global impact on the commercialization of consumer products. 3D printers range from large industrial devices to smaller printers that are typically used in homes and schools to provide a wide range of products, including toys, shoes, electronic equipment, automobile parts, and firearms. As these devices become more ubiquitous, questions are arising regarding the health and safety implications and how to mitigate any potential health risks to 3D printer users and consumers of products manufactured through these emerging processes, especially sensitive receptors such as children. The types of base materials used in 3D

printers include thermoplastics, metals, nanomaterials, polymers, and volatile and semi-volatile organic chemicals. The printing process may take several hours, and during this time period, the base materials are subjected to high heat (e.g., 220°C), resulting in a range of chemical by-products and particulates that may be released into indoor environments. Given these unknowns, scientist have begun to conduct studies to characterize and quantity these releases and their specific composition, particle size, and residence time in the indoor environment and produce data that can ultimately be incorporated into robust exposure assessments. *In vitro* and *in vivo* studies the toxicity of the base materials and reactive by-products and particulates. This session will include leading researchers who are conducting critical investigations into the release of compounds of concern from 3D printers and their toxicity potential. This Workshop session will provide an overview of additive manufacturing/3D printers and some key factors associated with these devices that may impact release of and exposure to chemicals of concern. Included in this analysis is the impact of device- and feedstock-related factors that influence emissions, providing important insights on exposure potential, efficacy of control technologies, and design of experimental toxicology studies. The session will describe the impact of ABS thermoplastic emissions on the pulmonary system of rats and their implications for human health, in addition to the release of volatile organic compounds (VOCs) as well as particles and the need to mitigate exposures to these compounds.

1054 Inhalation Toxicity of Acrylonitrile Butadiene Styrene (ABS) 3D Printer Emissions in Rats

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Fused filament fabrication 3D printing with acrylonitrile butadiene styrene (ABS) filament emits billions of particles and numerous volatile organic compounds (VOCs). This study sought to investigate the toxicity of ABS emissions from a 3D printer both *in vivo* and *in vitro*. For the *in vivo* studies, Sprague Dawley rats were exposed to real-time ABS printing emissions or air (control) for 4 h/day, 4 days/week for 1, 4, 8, 15, and 30 days. The average aerosolized particle concentration was 0.24 ± 0.09 mg/m^3, and the average median particle electric mobility diameter was 85 nm with an average geometric standard deviation of 1.6. Benzene was the predominant VOC released during printing. At 24 h after the last exposure, rats were assessed for pulmonary injury, inflammation, and oxidative stress as well as systemic and other organ toxicity. Results showed that among the measured cytokines in bronchoalveolar lavage, only IL-10 and IFN-γ at day 1 and 4, and IL-13 at day 30, of the exposure were increased when compared with the air-control. Moreover, neither pulmonary oxidative stress responses nor histopathological changes of the lungs were found among the exposed rats. There were no significant differences in serum cytokines levels or hematological indices, except for an increase in platelets and monocytes at day 15. Several serum biomarkers involved in liver damage were significantly higher at day 1 of the exposure. For the *in vitro* study, both particles and VOCs were collected into serum-free cell culture medium using an impinger sampler inside a chamber while printing for 1.5 h, followed by characterization of the physicochemical properties, as well as assessment of cytotoxicity, oxidative stress, and cytokine production in human small airway epithelial cells (SAEC). Results showed that particle numbers and VOC concentrations varied between print runs. Based on mixed model regression analyses, at 24 h post-exposure, ABS emissions induced significant dose-dependent cytotoxicity, oxidative stress, and production of proinflammatory cytokines in SAEC. In conclusion, our *in vitro* studies indicated that the emissions from ABS 3D printing induced toxicological effects, which were not substantiated by the *in vivo* studies with the current low exposure concentrations. Thus, more *in vivo* studies with higher dose-response are needed to verify the *in vitro* findings.

1055 3D Printer Emission Inhalation Impairs Systemic Microvascular Function

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We have previously reported that inhalation exposure to a variety of xenobiotic particles attenuates arteriolar dilation in the periphery and initiates an inflammatory response characterized by increased leukocyte trafficking. Three-dimensional printing has become routine in industrial, occupational, and domestic environments. Significant amounts of respirable emissions (3DPE) result from this process. We hypothesized that because both particles and gases are generated by this process, that after 3DPE inhalation exposure, the resultant systemic microvascular impacts would be more robust. Sprague Dawley rats were exposed to acrylonitrile butadiene styrene (ABS) 3DPE via whole body inhalation. The exposure parameters were: [3DPE] = 250 µg/m3; 4 hr/exposure; 1-30 days of exposure. The 3DPE mass median aerodynamic diameter was ~45 nm. This produced five calculated target 3DPE total lung burden groups: 2, 8, 16, 30, and 60 µg. Sham-control rats were exposed to filtered air only. Twenty-four hours after the last exposure, rats were anesthetized, and the mesentery was harvested for microvessel isolation. Second- and third-order arterioles were dissected and mounted on glass pipettes, perfused, and pressurized to assess endothelium-dependent (acetylcholine, ACh) and -independent (S-Nitroso-N-acetyl-DL-penicillamine, SNAP) vasoreactivity. Compared with sham-controls, 3DPE inhalation significantly attenuated arteriolar responsiveness to both ACh and SNAP. This was evident in a dose-response manner, and responsiveness was maximally inhibited at the 16 µg/rat lung burden. This 3DPE burden is ~50% less than that for a comparable effect after nano-titanium dioxide inhalation exposures. These results collectively support the notion that 3DPE inhalation exposures cause systemic microvascular dysfunction.

1056 Release of Aerosols from Fuse-Deposition Modeling 3D Printers and Associated Human Exposure

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Owing to a range of factors such as advancement in technology and increased affordability, 3D printers are gaining significant attention and popularization as a consumer product. Although there are many different techniques under the 3D printing definition, fuse-deposition modeling (FDM) consists of running a polymeric filament through a heated nozzle that moves to create the layers of a three-dimensional object. The high temperature of this nozzle causes gaseous and particulate air pollutants to be emitted in indoor environments, with compositions and concentrations that can vary significantly depending on the type of filament, nozzle temperature, printer type, and indoor environment characteristics, such as room size and ventilation rate. Another sparsely explored topic is the fundamental aerosol dynamics underlying particulate matter formation and growth during 3D printer operation, which is limited by instrumentation capability to measure the lower end of the particle size distribution. The objective of this work was to characterize aerosol emissions from the operation of a fuse-deposition modeling 3D printer. We characterized particle size distributions, optically absorbing particulate matter (PM), and total non-methane hydrocarbon emissions from a 3D printer in a chamber using a 1 nm SMPS, five wavelength aethalometer, and a flame-ionization detector, respectively. Effects of filament type on PM characteristics, emission rates, and emission factors were established. Our previous work showed that average aerosol emission rates ranged from ~108 to ~1,011 particles min-1, and rates varied over the course of a print job. Acrylonytrile-butadiene-styrene (ABS) filaments generated the largest number of aerosols, and wood-infused polylactic acid (PLA) filaments generated the smallest amount. Emission factors ranged from 6×108 to 6×1011 per gram of printed part, depending on the type of filament used. Ongoing work includes the application of a numerical model for particle formation (via nucleation) and growth to the experimental data for the estimation of the physical parameters of the semi-volatile emissions from different types of filaments. Lastly, model results will be used to estimate PM levels and resultant exposure when the same 3D printer is used in built environments with varying physical and ventilation characteristics.

1057 Factors Influencing Emissions from 3D Printers

A. Stefaniak. *NIOSH, Morgantown, WV.* Sponsor: Y. Qian

Additive manufacturing (AM), more commonly referred to as 3D printing, refers to several types of technologies that build physical objects layer by layer from a computer file. While some AM technologies remain limited to industrial settings, others, such as fused filament fabrication (FFF) and vat polymerization (VP), are relatively inexpensive and becoming more commonplace in homes, schools, libraries, and other nonindustrial settings. During operation, FFF and VP 3D printers emit particles and organic vapors. On a number basis, particles are dominated by ultrafine sizes (<100 nm) which can deposit in the lung alveoli. Organic vapors include compounds with known inhalation and dermal effects, including immune sensitization and toxicity. Both *in vitro* and *in vivo* data indicate that emissions may cause adverse health effects, including cytotoxicity and cardiovascular effects. Emissions are influenced by device-related (technology type, design, printing parameters) and feedstock-related (physical form, chemistry) factors. This presentation will summarize results from several years (and ongoing) of research on 3D printer emissions. Systematic investigation of device- and feedstock-related factors that influence emissions provides important insights on exposure potential, efficacy of control technologies, and design of experimental toxicology studies.

1058 Emissions from Consumer-Level 3D Printers

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Consumer-level fused filament fabrication (FFF) 3D printers are widely used in small-scale indoor environments and public spaces. Therefore, concern of potential health impacts of emissions from 3D printers has been raised, especially for vulnerable populations such as children. This study systematically characterized particle and volatile organic compound (VOC) emissions from multiple 3D printers using a standard test method. We found that 3D printing emits high concentrations of particles (especially ultrafine particles) and VOCs, the levels of which were associated with different print conditions like extrusion temperature, filament material, printer brand, filament brand, and color. The particle chemical compositions of 3D printer-emitted particles were similar to or different from the bulk filament material, indicating particle formations associated with the bulk material or additives in filament. A chemical assay (dithiothreitol, DTT) was applied to estimate oxidative potential of the particles emitted from 3D printers; exposure levels of some VOC species also were predicted using a model. Overall, emissions from 3D printing should be mitigated.

1059 The Role of LCN2 in Modulating the Inflammatory and Regenerative Pathways in APAP-Induced Acute Liver Failure

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Acetyl-para-aminophenol (APAP, Tylenol) is a commonly used analgesic medication that can be found as the main ingredient in over 600 different OTC and prescription medications. APAP overdose is one of the most common causes of acute liver failure (ALF) in USA. Following bioactivation by CYP3A4 and CYP2E1 enzymes, APAP generates a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI). Under normal doses, this reactive metabolite gets quenched into a non-toxic metabolite by cellular glutathione. However, following an overdose, the unquenched NAPQI covalently binds to liver macromolecules and causes centrilobular necrosis and sterile inflammation. Lipocalin-2 (LCN2) is an acute phase innate immune protein induced in the liver following injury. Recent data from our lab show that LCN2 KO mice are protected from ALF caused by APAP overdose when compared to WT mice. This protection was not due to differences in bioactivation based events but rather significantly less hepatocellular damage (histopathology and ALT) and higher liver regeneration (PCNA) at later stages (24-48h) post APAP overdose. Furthermore, we also demonstrated that LCN2 KO mice undergo increased autophagy as compared to WT mice. Preliminary studies done in our lab investigated the role of IL-17α and LCN2 in an APAP overdose model using enzyme linked immunosorbent assay (ELISA). This study found that LCN2 KO mice induced more IL-17α at 24 hours which correlated with the time point that showed a decrease in liver injury between WT and LCN2 KO mice. Our research suggests that regenerative pathways involving IL-17α may be inhibited in the presence of LCN2. The objective of this study is to investigate the mechanism of how LCN2 contributes to progression of injury in ALF. We hypothesize that LCN2 modulates the inflammatory and regenerative pathways via cytokines, prostaglandins and lipoxygenase pathways. The inflammation pathway will be investigated by measuring levels of 5-lipoxygenase and IL-1alpha in WT and LCN2 KO mice through ELISA. Prostaglandin E2 and IL-10 levels will be measured as anti-inflammatory markers. Regeneration pathways will be investigated by measuring levels of cyclooxygenase-2 (COX2), IL17α, and KI67 in WT and LCN2 KO mice. Findings from this study will identify a novel pathway in progressive phase of injury involving LCN2 and could potentially recognize targets for slowing down or inhibiting the progressive phase of liver injury that leads to ALF due to APAP overdose.

1060 Transfer of Hepatocellular microRNA Regulates Cytochrome P450 Enzymes in Renal Tubular Cells

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MicroRNA is released from cells injured by drug toxicity. In this study we tracked the transfer of the hepatocyte-specific microRNA, miR-122, between organs. Our hypothesis was that miR-122 will be transferred from liver to kidney as it is known that the kidney tubules can internalise microRNA containing extra-cellular vesicles. Dicer$^{flox/flox}$ mice were treated with an hepatotropic Cre recombinase-expressing adenovirus (AAV8-Cre) to inhibit microRNA production selectively in hepatocytes (conditional KO (CKO)). MicroRNA expression was analysed by PCR ± FACS sorting and by *in situ* hybridisation, with and without liver injury induced by acetaminophen (150mg/kg and 300mg/kg). A murine model of myocardial infarction was used as a non-hepatic model of microRNA release secondary to tissue injury. To determine the functional consequences of miR-122 transfer we measured the RNA and protein expression and drug metabolising activity of cytochrome P450 2E1 (established miR-122 target and key enzyme responsible for acetaminophen toxicity). In humans we measured microRNA expression in urinary extra-cellular vesicles. Dicer CKO mice had a time-dependent decrease in liver miR-122 (4 weeks post AAV8-Cre treatment, % of baseline miR-122: median 3% (IQR 2 - 6) N=20 P<0.0001). This was accompanied by a substantial decrease in kidney miR-122 (5% of baseline (IQR 2 - 7) N=20 P=0.007) without a change in kidney Dicer expression or other kidney microRNA species. There was no change in Dicer or microRNA in heart, lung or brain. Liver injury increased kidney miR-122 (9.5 fold increase, N=5, P=0.008); this increase was abolished in the Dicer CKO mouse (14 fold difference when AAV8-Cre compared to AAV8-null after 300mg/kg acetaminophen, N=5, P=0.008). FACS sorting demonstrated miR-122 uptake selectively into kidney tubular epithelial cells. Cardiac injury released circulating miR-499, which was also increased in the kidney. Depletion of hepatocyte miR-122 increased CYP2E1 expression and activity in the liver and kidney (2 fold increase in kidney mRNA expression, N=5, P=0.008). In human acetaminophen-induced hepatotoxicity, miR-122 was substantially increased in urinary extra-cellular vesicles (~500 fold). In summary, in normal physiology miR-122 is transferred from liver to kidney and this signalling pathway is up-regulated following hepatotoxicity. Kidney enzyme regulation by liver microRNA represents a new paradigm in drug metabolism.

1061 Mitochondrial Genetics Confer Differences in Susceptibility to Tolcapone Toxicity

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Idiosyncratic drug-induced liver injury (iDILI) has the potential to pose significant risk to patient health. Tolcapone, was developed for the treatment of Parkinson's disease but was withdrawn from the market due to cases of acute liver failure. Tolcapone is also a known mitochondrial uncoupler. Drug-induced mitochondrial toxicity (DIMT) is an important determinant of iDILI and differences in mitochondrial DNA (mtDNA) could account for the interindividual variation associated with iDILI. Therefore, transmitochondrial cybrids were employed to quantify the effect of mitochondrial haplogroup upon cell sensitivity to tolcapone and also to elucidate the mechanisms underlying such effects. Transmitochondrial cybrids were generated by fusing HepG2 rho-zero cells (devoid of mtDNA) with fresh platelets of a known mtDNA haplogroup, as anucleate mitochondrial donors. Cybrids allow the effects of mtDNA to be assessed against a stable nuclear background. Initial research employed the acute metabolic modification assay to compare the sensitivity of cybrids belonging to haplogroup H and J against HepG2 cells (haplogroup B). Subsequently, further mechanistic investigations were performed including changes in mitochondrial membrane potential (MMP), superoxide levels, mitochondrial morphology and dynamics. Comparison of the IC_{50}ATP values after 2 hours revealed that haplogroup J was significantly more susceptible to tolcapone-induced ATP depletion than haplogroup H (77.8 μM vs. 119.6 μM). Haplogroup J was also more susceptible to MMP loss in both whole cells and isolated mitochondria. However, following 24 hours, haplogroup H was more susceptible to tolcapone-induced ATP depletion than haplogroup B (39.1 μM vs. 86.2 μM). There was a significant difference between the IC_{50}ATP values at 2 hours and 24 hours for haplogroup H but not for haplogroup J and B. Overall, this research has shown that there are complex mechanistic differences in the susceptibility to tolcapone-induced toxicity dependent on mitochondrial haplogroup. The elucidation of compensatory mechanisms of mitochondrial protection within certain haplogroups has the potential to revolutionise Parkinson's disease via the stratification of tolcapone treatment. This mechanistic knowledge is not only valuable for Parkinson's disease but for all testing in which mitochondrial toxicity has been identified.

1062 Sex Differences in Bile Acid Metabolism

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Bile acids (BAs) are diverse signaling molecules that play important roles in lipid and glucose homeostasis, energy expenditure, inflammation, liver and gastrointestinal functions, and bacterial proliferation. There are currently over 30 different BA species known in humans and rodents. These BAs are

capable of interacting to varying degrees with many different receptors, including the farnesoid X receptor (FXR), vitamin D receptor, pregnane X receptor, G protein-coupled BA receptor 1, and sphingosine 1-phosphate receptor 2. However, the full functions of each individual BA *in vivo* remain unclear. Cyp7a1 and Cyp27a1 initiate the two main pathways of BA synthesis, the classical and the acidic pathways, respectively. We have previously reported on the generation and phenotypical characterization of male mice lacking these two key enzymes (double knockout---DKO) by crossbreeding *Cyp7a1*$^{-/-}$ and *Cyp27a1*$^{-/-}$ mice. These mice could be very valuable for the investigation of the differential functions of individual BA species *in vivo*. In order to assess any pharmacological or toxicological differences between sexes in BA signaling, we now characterized female mice deficient in genes *Cyp7a1* and *Cyp27a1*. In the current study, plasma, liver, gallbladder, and intestines were collected from 3-4 month old female wild type (WT), *Cyp7a1*$^{-/-}$, *Cyp27a1*$^{-/-}$, and DKO mice for LC-MS analysis of BA profiles. Additionally, mice were treated with GW4064, a synthetic FXR agonist, to determine if the DKO female mice had an altered responsiveness to FXR activation. We have found that female WT mice had significantly higher plasma BAs than their male counterparts, which is in line with known sex differences. Female *Cyp7a1*$^{-/-}$, *Cyp27a1*$^{-/-}$, and DKO mice had reductions in plasma BAs of 65.9%, 83.1%, and 62.9% respectively, as compared to WT mice. Despite having significantly less BAs than female WT mice, female DKO mice did not have a significant difference in hepatic mRNA expression of genes involved in BA synthesis (Cyp7b1, Cyp8b1), transport (*Slc10a1*, *Abcb11*), or regulation (Shp, Fxrα). Additionally, DKO mice maintained responsiveness to FXR agonism as measured by the mRNA expression of classical FXR response genes (Cyp8b1 and Shp). In summary, the two genes, *Cyp7a1* and *Cyp27a1*, are critical in both male and female mice for BA homeostasis. Together with the male DKO mice, the characterization of female DKO mice can provide a useful tool in the investigation into the differential effects of individual BAs between sexes. *Funding: NIH ES029258, ES007148, DK122725.*

PL 1063 Protease-Activated Receptor-1 Expressed by Hepatic Stellate Cells Drives Coagulation Activation and Fibrosis in Experimental Chronic Liver Injury

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Chronic hepatic injury is associated with activation of blood coagulation. Prior studies suggest that activation of coagulation leads to increased thrombin activity, which in turn drives hepatic fibrosis by activating protease-activated receptor-1 (PAR-1) expressed by stellate cells. However, because PAR-1 is expressed by numerous cell types, the specifics of this pathway are largely assumed. We used novel mice to define the precise role of PAR-1 expressed by stellate cells in experimental hepatic fibrosis. Male mice with a stellate cell-specific deletion of PAR-1 (PAR-1$^{flox/flox}$/LRATCre mice) and wild-type littermates (PAR-1$^{flox/flox}$) were challenged twice weekly with carbon tetrachloride (CCl_4, 1 mL/kg i.p.) for 6 weeks. Stellate cell activation and hepatic fibrosis, indicated by α-smooth muscle actin (αSMA) labeling and hepatic collagen staining, were significantly reduced in PAR-1$^{flox/flox}$/LRATCre mice. Importantly, the reduction in fibrosis was not explained by differences in acute CCl_4-induced hepatocellular necrosis. However, to our surprise, stellate cell PAR-1 deficiency dramatically reduced coagulation activation after CCl_4 challenge, as indicated by plasma thrombin-antithrombin complex levels. Consistent with the literature, stimulation of isolated wildtype mouse hepatic stellate cells with PAR-1 agonist peptide had only modest effect on markers of myofibroblast transition. However, PAR-1 activation caused robust induction of mRNA encoding tissue factor (TF), the primary activator of blood coagulation. This effect was synergistically enhanced by treatment with the profibrogenic mediator transforming growth factor-β1. Furthermore, PAR-1 agonist peptide significantly increased TF procoagulant activity in cultured stellate cells. The results are the first to definitively document a role for hepatic stellate cell PAR-1 in experimental hepatic fibrosis. Moreover, the results suggest a novel mechanism whereby stellate cell PAR-1 amplifies intrahepatic coagulation by upregulating TF expression.

PL 1064 An Endocrinized Fibroblast Growth Factor 1 Variant Reverses Nonalcoholic Fatty Liver Disease via Activating AMPK in Type 2 Diabetes

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Nonalcoholic fatty liver disease (NAFLD) is common in type 2 diabetes (T2D) patients. Our recently engineered FGF1 partial agonist (FGF1$^{\Delta HBS}$) exhibits reduced proliferative potential, while preserving the full metabolic activity of native FGF1. This study showed that administration of FGF1$^{\Delta HBS}$ to 2-month-old db/db mice for 2 months lowered blood glucose, improved insulin sensitivity, lowered liver weight, lipid deposition, and inflammation and improved liver function. Simultaneously, FGF1$^{\Delta HBS}$ prevented diabetes-induced oxidative stress, promoted nuclear translocation of antioxidant transcription factor Nrf2 and elevated downstream antioxidant genes. In addition, FGF1$^{\Delta HBS}$ inhibited activity and/or expression of hepatic lipogenic genes SREBP-1, FAS and SCD-1. Furthermore, FGF1$^{\Delta HBS}$ restored hepatic fatty acid oxidation signaling including elevated CPT-1α, PPARα and PGC-1α, increased phosphorylation of AMPK along with AMPK substrates ACC1 and SREBP1. This demonstrates association of FGF1$^{\Delta HBS}$ modified lipid metabolism with activation of AMPK signaling. Mechanistically, hepatic cells treated with palmitate mimicked T2D phenotype of hepatic oxidative damage and lipid disorder, all of which could be reversed by supplementing cells with FGF1$^{\Delta HBS}$. Nrf2 knockdown by SiRNA abolished anti-oxidative actions of FGF1$^{\Delta HBS}$ but did not affect FGF1$^{\Delta HBS}$ actions on lipid metabolic disorder. Whereas, AMPK inhibition, using Compound C or SiRNA knockdown, abolished both the ability of FGF1$^{\Delta HBS}$ to prevent lipid metabolic disorder and produce antioxidative protection. Remarkably, 3 months administration of FGF1$^{\Delta HBS}$ to 9-month-old db/db mice completely reversed late NAFLD and activated Nrf2 and AMPK pathways. Most importantly, liver-specific AMPK knockout almost completely abrogated the preventive effects of FGF1$^{\Delta HBS}$ on NAFLD induced by HFHS diet. Our findings indicate that in addition to glucose-lowering and insulin-sensitizing effects, FGF1$^{\Delta HBS}$ can reverse NAFLD in T2D by activating AMPK, which prevents hepatic lipotoxicity by inhibiting lipogenesis and activating lipid oxidation.

PL 1065 Human CYP2B6 Is an Anti-Obesity Enzyme That Produces Active *a*-Linolenic Acid Metabolites

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Multiple factors in addition to overconsumption are believed to lead to obesity and non-alcoholic fatty liver disease (NAFLD) in the United States and elsewhere. Cyp2b-null mice that lack the primarily hepatic Cyp2b enzymes are diet-induced obese, primarily due to increased white adipose tissue, but also contain increased liver triglycerides. Therefore, studies were undertaken to determine whether human CYP2B6 is anti-obesogenic and a putative mechanism. Vivid human CYP2B6 recombinant enzyme was used to determine inhibitors of CYP2B6. Several polyunsaturated fatty acids (PUFAs) including arachidonic acid, linoleic acid, DHA, and α-linolenic acid (ALA) were inhibitors with EC50's below 10μM. LC-MS/MS revealed that CYP2B6 metabolized all PUFAs tested; however, there was a greater than 20-fold preference for metabolism of to 9-HOTre and to a lesser extent 13-HOTre, monohydroxylated products of ALA. This data indicates that CYP2B6 metabolizes a specific PUFA (α-linolenic acid) to a specific metabolite (9-HOTre) with unknown function. Interestingly, cellular incorporation of Nile Red, a triglyceride sensor, is greater in ALA-treated CYP2B6-transfected HepG2 cells than untransfected HepG2 cells, indicating that metabolism of ALA by CYP2B6 increases hepatic lipid accumulation, the opposite of what was expected. Increased lipid localization in ALA-treated CYP2B6-HepG2 cells was confirmed and quantified using confocal microscopy. We recently developed a humanized CYP2B6 mouse on our Cyp2b-null background and provided a 60% high-fat diet to hCYP2B6-Tg and Cyp2b-null mice for 16 weeks. The presence of CYP2B6 reduced weight gain and metabolic disease as measured by glucose tolerance tests, however hCYP2B6-Tg male mice showed increased liver triglycerides, consistent with the data described in the CYP2B6-HepG2 cells. Currently, we are identifying changes in serum oxylipins and hepatic ALA metabolism between hCYP2B6-Tg and Cyp2b-null mice. In addition, several pharmaceuticals and environmental chemicals were screened using the Vivid CYP2B6 recombinant enzyme. Triclosan, diazinon, endosulfan, atrazine, ticlopidine, parathion, chlorpyrifos, jet fuel, nonylphenol, and others were found to be CYP2B6 inhibitors. Overall, this study defines a mechanism by which chemical inhibition of CYP2B6 can increase diet-induced obesity and metabolic disease through reduced production of ALA metabolites and disruption of lipid distribution between the liver and other tissues.

1066 The Sulfate Metabolite of 3,3′-Dichlorobiphenyl (PCB-11) Impairs Cyp1a Activity and Increases Hepatic Neutral Lipids in Zebrafish Larvae (*Danio rerio*)

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3,3′-Dichlorobiphenyl (PCB-11) is a lower-chlorinated PCB congener byproduct of diarylide pigment manufacturing, and PCB-11 and its metabolites are detected in human samples. Zebrafish (*Danio rerio*) embryo exposures to 20 µM PCB-11 impair lipid metabolism-related genes, and in co-exposures with the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) inhibits Cyp1a function to exacerbate cardiovascular and craniofacial malformations. In this study, two prevalent PCB-11 metabolites, OH-PCB-11 and PCB-11-Sulfate, were tested in acute exposures to understand if they drive Cyp1a effects observed with PCB-11, and in low concentration chronic exposures to understand if they increase hepatic neutral lipid accumulation. In acute experiments, wildtype AB embryos were statically exposed to 0.02-20 µM OH-PCB-11 or 0.2-20 µM PCB-11-Sulfate from 1-4 days post fertilization (dpf) and the EROD bioassay was used to assess Cyp1a activity, with and without co-exposures to 100 µg/L B[a]P. In chronic experiments, fish were exposed to 0.2 µM of either parent or metabolite compounds from 1-15 dpf, and in a subsequent experiment co-exposed to 10 µg/L B[a]P and 0.002-0.2 µM PCB-11-Sulfate; survival, larval growth, and hepatic lipid accumulation was assessed using Oil-Red-O staining. For acute experiments, 2 and 20 µM OH-PCB-11 was lethal, but fish exposed to 0.02-0.2 µM OH-PCB-11 and 0.2-20 µM PCB-11-Sulfate developed normally with low Cyp1a activity. In co-exposure experiments, 20 µM PCB-11-Sulfate significantly lowered the Cyp1a activity of B[a]P, and these fish developed normally. For chronic experiments, lipid accumulation was significantly increased 30% in fish exposed to 0.2 µM PCB-11-Sulfate, and a trend towards a dose-dependent increase in lipid accumulation was observed in co-exposures with PCB-11-Sulfate and B[a]P, though not statistically significant. These findings indicate OH-PCB-11 is more acutely toxic than PCB-11-Sulfate, but that PCB-11-Sulfate can affect Cyp1a activity and increase hepatic neutral lipid accumulation. Further long-term hepatic studies would better elucidate the effects of this emerging contaminant, particularly in the context of environmentally-relevant mixtures.

1067 Health Effects from Freshly Emitted versus Oxidatively or Photochemically Aged Air Pollutants

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Epidemiology studies over the past five decades have provided evidence that exposure to air pollution is associated with multiple adverse health outcomes, including increased mortality. Air pollution is a complex mixture of particles, vapors and gases emitted from natural and anthropogenic sources as well as formed through photochemical transformation processes. In metropolitan areas, air pollutants from combustion emissions feature a blend of emitted particles, oxides of carbon, sulfur and nitrogen, volatile organic compounds (VOCs), and secondary reaction products, such as ozone, nitrogen dioxide, and secondary organic aerosols. Since many of the primary and transformed pollutants track together, their relative contributions to health outcomes are difficult to disentangle. Aside from the criteria pollutants ozone and nitrogen dioxide and some of the simpler aldehydes (e.g. formaldehyde and acrolein), other products from photochemical processes are a particularly vexing class of chemicals to investigate since they comprise a dynamic ill-defined complex mixture in both particulate and gas phases. The purpose of this poster is to describe and compare health effects of freshly emitted versus oxidatively or photochemically aged air pollutants from existing literature. In some cases, (e.g. single VOCs) photochemical transformation resulted in marked enhancements in respiratory toxicity and mutagenicity through formation of both known and unidentified reaction products, while in other examples (e.g. aging of automobile emissions) the potentiation of respiratory and cardiovascular effects was variable. The variation in experimental design, aging system used, concentration and type of starting agent, and toxicity endpoints make comparisons between different studies exceedingly difficult. A more systematic approach with a greater emphasis on higher throughput screening and computational toxicology is needed to fully answer under what conditions oxidatively- or photochemically-transformed pollutants elicit greater health effects than primary emissions. *The views presented in this document are those of the authors and do not necessarily represent the views or policy of the US EPA.*

1068 Volatile Organic Compound Exposures Are Positively Associated with Liver Apoptosis in a Residential Cohort

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More data are required on the potential impact of volatile organic compound (VOC) exposures on liver health in community residents. The actively enrolling Health Environment and Action in Louisville (HEAL) study will determine associations between residential VOC exposures and cardiometabolic syndrome. The objective of this interim cross-sectional analysis of the first 391 HEAL subjects is to determine associations between urinary VOC metabolites (exposure biomarkers) and the circulating hepatocyte apoptosis biomarker, caspase-cleaved keratin 18 (K18 M30, disease biomarker). Consenting subjects were enrolled in HEAL. Serum K18 M30 (normal cutoff <200 U/L) was determined by ELISA. 15 creatinine-adjusted urinary metabolites of 10 parent VOCs were measured by UPLC-MS^2. Generalized linear models were used to test for associations between disease and exposure biomarkers. Final models were adjusted for race, BMI, alcohol use, cotinine-confirmed smoking status, and education level. Interactions were included in the models, followed by subgroup analysis to determine whether associations were modified by demographic characteristics. Final results are presented as % change (95% CI) per interquartile range of VOC metabolite. Results: Mean age was 50.3±12.0 (SD). Mean BMI was 29.9±6.1 kg/m^2. The cohort was 57.8% female, 78.5% white and 22.0% diabetic. Elevated K18 M30 was present in 12.3% of participants. K18 M30 was positively associated with 4 urinary VOC metabolites corresponding to acrolein (3HPMA); acrylamide (AAMA); and 1,3-butadiene (DHBMA and HPMMA) exposures. These associations were modified by sex and by race for three of the four significant VOC metabolites each; and were more positive in males and whites. This interim analysis demonstrated positive associations between specific VOC exposures and hepatocyte apoptosis in the residential HEAL cohort. Race and sex modified these effects. The role of VOC exposures in metabolic liver diseases warrants further investigation. HEAL is still recruiting and all subjects will be analyzed in the future.

1069 *Caenorhabditis elegans* as a Sensor of Urban Dust Toxicity in a Caribbean City

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Toxic substances from various sources can be associated with the surface of particulate matter suspended in air, forming a heterogeneous mixture that settles as dust. Fine particles of urban dust have high environmental mobility and elevated adsorption capacity for pollutants, representing a hazard health for residents. The objective of this study was to evaluate the toxic effects of sedimented dust extracts from the urban area of Barranquilla (Colombia), the largest Colombian Caribbean city, in *Caenorhabditis elegans*. Urban dust samples (<75 µm) were collected at thirty-five points distributed in five localities, including industrial areas, ports, and high traffic. Aqueous extracts (K-medium) were obtained from dust particles and evaluated on the nematode, measuring lethality, growth, locomotion, and gene expression with GFP transgenic strains. Mercury was determined by direct Hg analysis. Urban dust extracts induced a maximum lethality of 98.8%, with greater toxicity in areas known as Riomar, Metropolitana and Suroriente. Interestingly, nematode size increased in 71.4% of the samples, while locomotion was inhibited up to 61.8%. The highest expression of *mtl*-2, *sod*-4, and *unc*-25 genes was observed after 6 h of exposure. The concentration of Hg ranged between 5 and 139 ppb; however, it did not correlate with any biological output. Lethality displayed a positive association with gene expression for *mtl*-2 ($r = 0.337$, $p = 0.048$) and *unc*-25 ($r = 0.411$; $p = 0.014$), and a negative correlation with locomotion ($r = -0.336$, $p = 0.049$) and growth ($r = -0.523$, $p < 0.01$). In short, *C. elegans* is a sensitive organism capable of responding to the exposure of urban dust particle extracts, being a suitable biosensor for the implementation of warning systems associated with human health risks linked to air pollution. *Colciencias-Unicartagena, 785-2017. Vice-Rectory for Research, 2018-2019.*

1070 Potential Role of Ferroptosis in Toxicity Induced by 9,10-Phenanthrenequinone in Human Lung Epithelial Calu-1 Cells

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9,10-Phenanthrenequinone (9,10-PQ), which is a redox-active polycyclic aromatic hydrocarbon found in diesel exhaust particles and cigarette smoke, plays a crucial role in the adverse health effects triggered by air pollution. The toxicity of 9,10-PQ has been associated with the generation of reactive oxygen species (ROS) via the process of redox cycling. In the present studies, we investigated mechanisms of 9,10-PQ induced toxicity in human lung epithelial Calu-1 cells. 9,10-PQ treatment caused a time- and concentration-dependent decrease in cell viability. This was associated with a decrease in intracellular glutathione (GSH) and glutathione peroxidase (GPX) activity, 9,10-PQ caused an increase in the ratio of GSSG to GSH. 9,10-PQ also increased levels of intracellular ROS and lipid peroxidation products in a concentration- and time-dependent manner, as measured by the CM-H_2DCFDA and BODIPY assays, respectively. These data indicate that 9,10-PQ caused oxidative stress in Calu-1 cells. N-acetyl cysteine (NAC) and β-mercaptoethanol (β-ME) pretreatment inhibited 9,10-PQ induced ROS generation and partially suppressed 9,10-PQ-induced cell death. Regulated cell death characterized by the iron-dependent accumulation of lipid peroxides, referred to as ferroptosis. Ferrostatin-1, a lipid ROS scavenger and specific inhibitor of ferroptosis, and deferoxamine mesylate (DFO), an iron (III) chelator, were found to decrease 9,10-PQ induced ROS production and protect Calu-1 cells from toxicity. In contrast, z-VAD-FMK, a pan-caspase inhibitor, had little or no effect on 9,10-PQ toxicity, indicating caspase-mediated apoptosis was not involved in the actions of 9,10-PQ. Taken together, these data indicated that ferroptosis is a critical cell death pathway induced by 9,10-PQ. *Support: NIH grants AR055073, NS108956, ES004738, and ES005022.*

1071 *In Vitro* Cytotoxicity and Potential Carcinogenesis of Copper Oxide Nanoparticles

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Occupational exposures to copper nanoparticles and fine particles have been reported to be harmful to human health. There is a possible risk of cancer among industrial workers. Copper (II) oxide (CuO) nanoparticles have not, to date, been extensively examined for potential carcinogenic effects. Mechanisms of CuO-induced pathogenesis, the effect of CuO nanoparticles on cyto-toxicity and carcinogenic effects were investigated. Using the 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide cellular dehydrogenase activity (MTT Assay) assay, CuO nanoparticles showed a dose-dependent reduction of the dehydrogenase activity (DHA) in the JB6 cell culture medium indicating the cytotoxicity of CuO. The potential carcinogenesis effects of CuO were also examined. The data indicates that chronically exposed human lung epithelial JB6 cells to low-dose CuO resulted in neoplastic transformation using the soft agar assay. *In vitro* genotoxicity test, single cell gel electrophoresis assay (SCGE; also called the comet assay), was used for the detection of the mutagenic potential of nano- and fine-sized CuO. The results suggested that both nano- and fine-sizes CuO induce DNA damage. CuO nanoparticles exhibit greater genotoxicity than fine particles. Understanding complex mechanisms associated with these events may provide insights into the initiation and progression of CuO-induced carcinogenesis.

1072 Serum-Borne Factors Alter Cerebrovascular Endothelial microRNA Expression following Particulate Matter Exposure Near an Abandoned Uranium Mine on the Navajo Nation

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Commercial uranium mining on the Navajo Nation has left communities on tribal lands in the Southwestern United States with a number of health effects from exposure to residual environmental contamination. There is an association between residential mine-site proximity and circulating biomarkers in residents, however the contribution of mine-site derived wind-blown dusts on vascular and other health outcomes is unknown. To assess neurovascular effects of mine-site derived dusts we exposed mice using a novel exposure paradigm, the AirCARE1 mobile inhalation laboratory, located 2 km from an abandoned uranium mine, Claim 28 in Blue Gap Tachee, AZ. Mice were exposed to filtered air (FA) (n=6) or $PM_{2.5}$ (n=5) for 2 weeks for 4 hours per day. To assess miRNA differential expression in the cerebrovasculature following PM exposure, the serum cumulative inflammatory potential (SCIP) assay was employed. MiRNA sequencing was then performed in mouse cerebrovascular endothelial cells (mCECs) to evaluate transcriptional changes. The most significantly altered miRNAs were validated in the serum. Results indicated 27 highly differentially expressed ($p<0.01$) murine miRNAs following the SCIP assay. Of the 27 highly differentially expressed murine miRNAs, 9 of them (~30%) were significantly altered in the serum and 8 of those demonstrated the same directional change (either upregulation or downregulation) as cellular miRNAs following the SCIP assay. Our data suggest that miRNAs from the systemic circulation may translocate to the endothelium following $PM_{2.5}$ exposure, as approximately 30% of highly differentially expressed cellular miRNAs were validated in the serum. While translocation of miRNAs via exosomes or an alternative mechanism is certainly possible, other yet-to-be-identified factors in the serum may be responsible for significant miRNA differential expression in endothelium following inhaled exposures. Additionally, the most highly upregulated murine miRNAs in the $PM_{2.5}$ exposure group were in the let-7a family. These miRNAs play a prominent role in cell growth and differentiation and our data suggest that mmu-let-7a may contribute to BBB disruption following inhaled dust exposure.

1073 Respiratory Impacts of Hazardous Air Pollutants in Allergic Mice: Sex Differences and *In Vitro* Concordance

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Validation of high-throughput *in vitro* assessments of hazardous air pollutants (HAPs) and other EPA high-priority chemicals requires testing in animal models to demonstrate *in vitro* predictive ability. Acrolein (ACR) and trichloroethylene (TCE) are among several HAPs designated as high priority chemicals within the US EPA Toxic Substances Control Act inventory, and may exacerbate respiratory symptoms in susceptible populations such as asthmatics. Here we evaluated real-time pulmonary responses to ACR and TCE in control and house dust mite (HDM)-allergic male (M) and female (F) mice and subsequent inflammatory biomarkers. On 2 consecutive days, mice were exposed nose-only in head-out plethysmographs to air (20 min) followed by increasing concentrations (25 min each) of ACR (0.1, 0.32, 1.0, 3.2 ppm) or TCE (3.2, 10, 32, 100 ppm), corresponding to ongoing *in vitro* assessments. Separate groups were exposed to air only to compare inflammatory responses. Breathing frequency significantly declined in both M and F HDM-allergic groups at 1.0 ppm ACR on both exposure days (group means 19-30% lower than air baseline) and was sharply lower (39-67%) at 3.2 ppm ACR, while TCE (10-100 ppm) also significantly reduced frequency, though to a lesser extent (12-23%). M mice had higher baseline frequency than F, which declined to a greater extent with increasing ACR concentration. Other parameters of respiratory timing, flows, and volumes indicated overall greater effects of ACR and TCE in M and allergic groups compared with F and control groups. Four hours after final exposure, HDM-allergic F mice had greater indices of allergic inflammation than M mice (e.g. bronchoalveolar lavage (BAL) eosinophils, lymphocytes, albumin, and IL-5), but relatively few differences with respect to HAPs exposure. TCE-exposed HDM-allergic F mice had greater BAL eosinophils and N-acetylglucosaminidase, but lower MIP-2, than ACR-exposed allergic F mice. Effects of ACR and TCE on real-time respiratory physiology correlated reasonably well with cell viability results in BEAS-2B cells (CellTiter Glo viability ATP assay). Assessment of additional HAPs in the allergic mouse model will further determine validity of HAPs effects predicted by high-throughput *in vitro* assays. *This abstract does not represent US EPA policy.*

1074 Plasma from C57BL/6 Mice Exposed to Vehicle Emissions Mediates Alterations in Lipid Accumulation and Renin-Angiotensin Pathway Signaling in 3T3-L1 Mouse Adipocytes

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In addition to cardiopulmonary disease, exposure to traffic-generated air pollutants has been implicated recently in the pathogenesis of metabolic disorder and obesity. We have previously reported that inhalation exposure to mixed vehicle exhaust (MVE) results in increased the levels of circulating angiotensin II (Ang II), associated with adipose fat pad weight, increased adipocyte size, and lipid accumulation in C57Bl/6 wild type mice, which is further exacerbated by consumption of a high fat (HF) diet. To investigate mechanisms involved in MVE-mediated alterations in adipocytes, we examined whether circulating factors in the plasma of MVE-exposed mice mediate alterations of the renin-angiotensin system (RAS) signaling or lipid accumulation in adipocyte 3T3-L1 cell culture. Three mo-old male C57Bl/6 mice on either an HF or low fat (LF) diet randomly assigned to inhalational exposure of either filtered-air (FA) or a mixture of 30 μg PM/m^3 gasoline exhaust + 70 μg PM/m^3 diesel exhaust (MVE) for 6 hr/d for 30 d; plasma was collected at the end of the study and was applied to the media of the adipose cell culture and incubated for 48 hours. Adipocytes were measured for cell area, lipid accumulation by Nile Red staining, and RAS member gene expression, by qRT-PCR. Our results show that exposure to MVE + HF diet resulted in a significant increase in cell size and cellular lipid accumulation, associated with significantly increased expression of angiotensinogen (AGT), angiotensin II type 2 receptor (AT_2), and peroxisome proliferator-activated receptors gamma (PPARγ) mRNA. Pretreatment of the adipocytes with an AT_1 receptor antagonist, Losartan, before incubation with the plasma from the exposure study resulted in a significant decrease in AGT, AT_2 receptor, and PPARγ in MVE+HF mice. Thus, our results suggest that exposure to traffic-generated pollution with a concurrent consumption of an HF diet high fat leads to alterations of adipocyte signaling and lipid accumulation associated with metabolic syndrome. Moreover, these alterations appear to be mediated (at least in part) through Ang II -AT_1 signaling pathways. *Funded by NIEHS R15ES026795 to AKL.*

1075 Top-Down Proteomics Reveals Alterations in the Liver Protein Profiles of C57Bl/6 Mice with Inhalation Exposure to Mixed Vehicular Emissions and a High-Fat Diet

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Traffic-generated emissions are known to induce cardiovascular events, compromise neurological systems, and alter cellular responses and function, many of which are exacerbated when coupled with consumption of a high-fat diet. It has been well documented that exposure to environmental air pollutants can mediate alterations in hepatic transcript and protein expression, which can result from biotransformation reactions in the hepatocytes. Additionally, exposure-mediated alterations of protein expression in the liver are known to contribute to the pathogenesis of diseases in the liver, such as non-alcoholic fatty liver disease (NAFLD), which has been associated with air pollution-exposure in adults and children. Thus, we investigated the hypothesis that exposure to a ubiquitous environmental air pollutant, vehicle emissions, coupled with concurrent consumption of a high fat (HF) diet, significantly alters proteome profiles in the liver. To test this hypothesis, 3 mo-old male C57Bl/6 mice were placed on either a low fat or high fat (HF: 21% fat content by weight), and then assigned to be exposed by whole body inhalation to either filtered air (FA) or mixed gasoline and diesel engine emissions (MVE: 70 μg PM/m^3 diesel exhaust + 30 μg PM/m^3 gasoline exhaust) for 6 hr/d for 30d. Liver tissue was collected, homogenized, and analyzed via top-down proteomics utilizing a Waters XBridge Protein BEH C_4 column installed on an ACUITY UPLC coupled with a SYNAPT G2 q-TOF. Isolated protein samples were processed using a mobile phase gradient (10-90% acetonitrile: water) with 0.1% trifluoroacetic acid. Chromatogram data point display potential statistical expression differences (>20% compared to LF/FA controls) of 111 up-regulated and 138 down-regulated points in the livers from HF/FA animals, 142 up-regulated and 315 down-regulated points in the livers of LF/MVE animals, and 1013 up-regulated and 43 down-regulated points in the livers of HF/MVE animals, when compared to the livers from the LF/FA animals. Ongoing investigation and calculations are being performed to determine the identities of the altered proteins to better understand the milieu resulting from exposure to traffic generated air pollution, combined with an HF diet, in the liver that may contribute to the pathogenesis of diseases such as NAFLD. *Funded by NIEHS R00ES016586 and R15ES026795 (AKL).*

1076 Lipidomic and Epigenetic Alteration for Toxic Mechanisms of Air Pollution on Alzheimer's Disease

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Air pollution, particularly fine particulate matter, such as $PM_{2.5}$, targets various organs including brain. Therefore, we performed biomonitoring of air pollution with an exposure biomarker, urinary 2-naphthol (NT), and compared lipidomic and epigenetic alterations between 5 Alzheimer's disease (AD) patients and 15 controls (N=20; age=60.75±13.8; 9 men). We analyzed urinary 2-NT and malondialdehyde (MDA), an oxidative stress biomarker, with HPLC/FD and LC/MS/MS and blood C-reactive protein (CRP) and urinary creatinine with a clinical analyzer. Urinary 32 fatty acids were analyzed with GC/MS/MS. For epigenetics, global and specific methylation statuses were analyzed with EpigenTek kit for 5-mC and methylation specific PCR for DNA methylation at *MATP*, microtubule associated protein tau, respectively. As results, we found positive associations between urinary 2-NT levels and $PM_{2.5}$ in their residential areas and among urinary or plasma 2-NT, urinary levels of MDA, and blood levels of CRP (ps<0.05). When we adjusted for age, sex and tobacco smoking, plasma 2-NT levels were somewhat higher in AD patients than controls (OR=31.87, 95% CI=-1.07~3.67). We also found positive correlations between levels of urinary 2-NT and some saturated fatty acids, such as myristoleate or pentadecenoate. In addition, the levels of urinary arachidonic acid involved with tau phosphorylation was associated with *MAPT*-DNA methylation (r^2=-0.15, p=0.04). Therefore, the above lipidomic and epigenetic results provide some potential risks of air pollution on AD.

1077 Toxicomultiomics Profiling: Pulmonary Toxicity Studies with Repeated Exposure of PHMG-p in Mice

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Polyhexamethylene guanidine phosphate (PHMG-p) was used as a humidifier disinfectant in Korea. PHMG induced severe pulmonary fibrosis in Koreans. The objective of this study was to elucidate mechanism of pulmonary toxicity caused by PHMG-p in mice using multi-omics analysis. Balb/c mice were intratracheally instilled with PHMG-p by 4-week (0, 0.03, 0.1 mg/kg, 2 times/week) repeated administration. Histopathologic examination of lung was performed with H&E staining. Inflammatory cell infiltration, multifocal, and minimal were observed in response to 0.03 mg/kg of PHMG-p. In addition, mononuclear cell infiltration, multifocal, minimal, fibrosis, and alveolar wall were observed in response to 0.1 mg/kg of PHMG-p. Next generation sequencing (NGS) was performed for transcriptome profiling after mRNA isolation from bronchiol-alveoli. Bronchiol-alveoli proteomic profiling was performed using an Orbitrap Q-exactive mass spectrometer. Plasma metabolites were determined using ^{1}H-NMR. Among up-regulated 47 DEGs and 46 DEPs, changes of 3 mRNA levels were significantly correlated with changes of their protein levels in response to 0.03 mg/kg of PHMG-p. Among up-regulated 643 DEGs and 132 DEPs, changes of 39 mRNA levels were significantly correlated with changes of their protein levels in response to 0.1 mg/kg of PHMG-p. In addition, among down-regulated 613 DEGs and 80 DEPs, changes of 7 mRNA levels were significantly correlated with changes of their protein levels in response to 0.1 mg/kg of PHMG-p. Remarkable biological processes represented by both DEGs and DEPs in response to 0.1 mg/kg of PHMG-p were response to stress, immune system process, defense response, response to external stimulus, and regulation of molecular function. In addition, up-regulated DEGs and DEPs associated with pulmonary fibrosis and asthma were selected in response to 0.1 mg/kg of PHMG-p, the major transcription factors associated with pulmonary fibrosis and asthma were 6 (Lgals3, Egfr, Arg2, Hmox1, Arg1, Serpinh1) and 8 (Lgals3, Spp1, Egfr, Arg2, Hmox1, Arg1, Lrg1, Tnc) species, respectively. And down-regulated DEGs and DEPs associated with pulmonary fibrosis and asthma were 1 (Scgb1a1) and 2 (Ank1, Scgb1a1) species, respectively. On the other hand, there were no DEGs and DEPs associated with pulmonary fibrosis and asthma in response to 0.03 mg/kg of PHMG-p. In metabolomics study, 13 and 23 metabolites (VIP> 0.5) were determined in plasma of response to 0.03 and 0.1 mg/kg of PHMG-p. And then, we performed an integrated analysis with selected metabolites from plasma after screening for DEGs and DEPs associated with pulmonary disease. Glutamate, glycine, creatine, acetate, histidine and choline were selected as major metabolites and were found to be major factors to pulmonary fibrosis and asthma in association with up-regulated DEGs and DEPs in response to 0.1 mg/kg of

PHMG-p. And down-regulated DEG and DEP related metabolites in the high dose group were glutamate, acetate, glycine and choline. Also, glycine, formate, citrate, glycerol, valine, isoleucine, and taurine were selected as major metabolites and were found to be major factors to pulmonary fibrosis and asthma in association with up-regulated DEGs and DEPs in response to 0.03 mg/kg of PHMG-p. Current study could be helpful for identifying toxicity and toxicological indicators caused by inhalation exposure of PHMG-p, a component of humidifier disinfectants.

PS 1078 Exposure to Diesel Exhaust Particles Alters Immune Response and Promotes the Expansion of Proteobacteria in the Lungs of C57BL/6 Mice

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Traffic generated emissions have been associated with the development of inflammatory lung disorders such as asthma and COPD. Healthy lungs were initially considered to be sterile, but there is evidence now that suggests the presence of microbes within the airways. A shift in the lung microbiota towards Proteobacteria is observed in multiple lung diseases. The effect of air pollutants on the lung microbiota is currently not well characterized. Although there is an increase in the incidence of lung disorders in heavily polluted regions, the pathogenesis of these diseases or the involvement of the lung microbiota remains largely unexplored. Thus, we investigated whether exposure to diesel exhaust particles (DEP) altered the immune response and microbiota profiles within the lung, as well as whether fat content in diet further contributed to the observed pulmonary outcomes. To do this, male C57BL/6 mice (4-6-week-old) were placed either on a low-fat diet (control) or a high-fat (HF) diet containing 45% fat. Mice were randomly assigned to be exposed via oropharyngeal aspiration to 35μg DEP, obtained from NIST (SRM #2975), suspended in 50μl 0.9% sterile saline or sterile saline only (control) twice a week for 30 days. Our results show that DEP-exposure significantly increased the production of IgA, as determined by ELISA. RT-qPCR analysis of cytokine profiles revealed increased pulmonary expression of inflammatory cytokines - TNFα and IL-10 in DEP-exposed animals vs. controls. Next-generation sequencing and qRT-PCR of bacterial 16S rRNA genes in BALF were performed to identify major bacterial groups. Overall microbial abundance in the lungs of DEP-exposed mice was decreased, with a significant reduction in Firmicutes observed in the DEP+HF diet animals. The relative abundance of *Enterobacteriaceae* belonging to the Proteobacteria phylum was found to be higher in the lungs from DEP + HF diet mice, compared to all other groups. Our results show that exposure to DEP causes a significant increase in the major lung immunoglobulin IgA suggesting the production of a robust immune response. Both pro-inflammatory, TNF-α and anti-inflammatory, IL-10 cytokine appear to be elevated with DEP-exposure. Such immunological changes within the lung likely increase nutrient availability, particularly nitrates, which favor the expansion of bacteria within the Proteobacteria phylum. These results suggest a potential mechanism by which air pollutants alter the lung microbiota, which may contribute to the pathogenesis of lung diseases.

PS 1079 Diesel Exhaust Particulate Matter Exposure Results in Altered Lipid Accumulation and Expression of Receptors Involved in Nonalcoholic Fatty Liver Disease

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Recent epidemiology studies report a positive correlation between exposure to traffic-generated air pollution and the pathogenesis of metabolic disorder and obesity. Nonalcoholic fatty liver disease (NAFLD), which often occurs concomitant with metabolic syndrome, is characterized by increased hepatic lipid accumulation and altered molecular signaling pathways involved in inflammation, fatty acid synthesis, and fibrosis. These pathways include signaling through the tumor necrosis factor receptor (TNFR1), adiponectin receptor (adipoR), and the oxidized LDL receptor (LOX-1), in addition to others. Furthermore, signaling through the aryl hydrocarbon receptor (AhR) is also reported to mediate the pathogenesis of NAFLD. We have previously reported that exposure to traffic-generated pollution is associated with increased adipose depots/weights and lipid accumulation in C57Bl/6 mice, which is further exacerbated by consumption of a high fat (HF) diet. To investigate the role of traffic-derived particulate matter (PM) on mediating detrimental hepatic outcomes related to NAFLD, 2 mo-old male C57Bl/6 mice on either an HF or low fat (LF) diet were exposed to either 35 μg diesel exhaust PM (DEP; 30 μl) or vehicle (saline) 2x/wk for 4 wk via OA. Livers were analyzed for lipid accumulation by Nile Red staining and transcript expression of TNFR1, adipoR, LOX-1 and AhR were analyzed by RT-qPCR. Nile Red staining revealed that DEP-exposure significantly increased hepatic lipid accumulation, which was further exacerbated in those on an HF diet. Additionally, transcript analysis showed a significant decrease in hepatic TNFR1 in DEP-exposed and HF-fed animals, compared to controls. AdipoR was significantly decreased in the livers of HF+DEP mice. Interestingly, we observed a significant decrease in hepatic LOX-1 in the HF+DEP group. DEP-exposure increased hepatic AhR mRNA in both the LF and HF diet groups. Thus, our results suggest that exposure to traffic-generated PM leads to alterations of hepatic lipid accumulation and signaling pathways associated with NAFLD and altered biotransformation. *Funded by NIEHS R15ES026795 to AKL.*

PS 1080 Characterizing Atmospheric Particle Toxicity from Biogenic and Anthropogenic Sources Using *In Vitro* Assays

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Plants emit highly reactive biogenic volatile organic compounds (BVOCs) that can contribute to production of secondary pollutants including secondary organic aerosols (SOA). There are many gaps in understanding how the interaction between biogenic and anthropogenic volatile organic compound (VOC) sources impact SOA yield, particle chemistry, and associated toxicity. While urban greening programs are directed to beautify urban areas and "filter out" atmospheric pollutants like particulate matter and tropospheric ozone, the chemical composition and toxic potential of plant emissions in urban versus clean environments remains an open question. SOA was generated in an oxidation flow reactor (Aerodyne, Inc) from isoprene, α-pinene, limonene, toluene and limonene/toluene mixtures. High oxidation and low oxidation conditions were compared, which mimicked oxidation reactions found in chemically pristine and polluted urban environments, respectively. The *in vitro* cytotoxicity of the produced particles was assed using murine lung macrophage from an established cell line. Cells were exposed to particles for four hours before assessing cell viability and oxidative stress. Cell health was assessed using the Lactate Dehydrogenase assay, reactive oxygen species formation was assessed using the Diogenes Superoxide assay, and nitric oxide release was assessed with the Griess Nitric Oxide assay. Changes in cell health and reactive oxygen species production were observed after exposure. Results show that SOA generated from a mixture of anthropogenic and biogenic sources was less toxic than SOA generated from either biogenic or anthropogenic precursors alone. Furthermore, SOA generated under lower oxidation conditions tended to be more toxic than SOA generated under higher oxidation conditions (as evaluated by O:C ratio). These findings suggest that plants can contribute to air pollution and that and specifics mixtures of VOC sources can impact atmospheric particle toxicity. Finally results from this type of study can be used to inform selections of plants used for urban greening programs, which may ultimately impact human health outcomes.

PS 1081 Advanced Age and a High-Fat Diet Potentiate Responses to Benzene Inhalation in Mice

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We have previously shown that mice inhaling volatile benzene (50ppm; 6h/day for 14 days) demonstrated tissue-specific insulin resistance. However, it is unclear if other risk factors for cardiovascular disease influence this outcome. To test this, we fed young (14wk) and old (24wk) mice normal chow or a high fat diet and exposed them to either filtered air or 50ppm benzene for 14d. At this time, insulin levels were measured and tissue levels of proteins and miRNAs impacting insulin signaling pathways were assessed. While young mice eating normal chow and exposed to benzene demonstrated a 1.5-fold increase in fasting plasma insulin compared to mice breathing filtered air, old mice on a high fat diet and exposed to benzene demonstrated a 4-fold increase in insulin levels compared to filtered air controls. Of several miRNAs known to target proteins involved in the regulation of insulin signaling, we observed that mir-125b was increased by 3.6-fold in the liver of high-fat diet, old mice inhaling benzene while this change was 0.9 fold in both old mice on normal chow, and young mice on a high fat diet. Consistently, hepatic levels of the catalytic subunit of PI3-kinase (PIK3CD), a target of mir-125b, were downregulated in high-fat, old mice inhaling benzene. In addition, levels of several miRNAs targeting phosphatase and tensin homolog (PTEN) were downregulated in high-fat, old mice inhaling benzene, but not in other mouse groups inhaling benzene. These results suggest that age and diet are factors that can exacerbate the consequences of benzene exposure and are consistent with the idea that multiple "hits" promote the toxicity of volatile organic compounds.

1082 Uptake and Toxicity of Respirable Uranium-Carbon-Bearing Particulate Matter in A549 Lung Epithelial Cells

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Understanding the toxic effects of respirable uranium (U)-bearing particulate matter (PM) with respect to the site-specific mineralogy is of great importance due to potential health impacts. In this study, U-carbon (C)-bearing particles were synthesized by mixing aqueous U with citrate to create an environmentally-relevant particles similar to PM found near Jackpile Mine, NM where U occurs in a highly enriched organic matter matrix. Cellular uptake and toxicity analyses were conducted following the exposure of the human lung epithelial cell line, A549 cells, to two groups of U-C-bearing particles (microparticles ≤ 10 μm and nanoparticles ≤ 0.6 μm) and to aqueous U at the same range of concentration (0-445 μM). Both microparticles and nanoparticles induced cytotoxicity in comparison to aqueous U which was dependent on U exposure concentration. After 2 hours exposure, microparticles showed four times higher U uptake than nanoparticles for cells exposed to 100 μM U whereas no U uptake was measured in cells exposed to aqueous U. TEM-EDS analysis confirmed the presence of both groups within vesicles of A549 lung cells but with no detectable adsorption on the cell membrane. Microparticles induced a higher DNA damage in A549 cells relative to nanoparticles and aqueous U. However, only nanoparticles showed an ability to interact with the cellular membrane by inducing hemolysis upon 24 h incubation with red blood cells. This study highlights the potential toxicological effects of respirable U-C-bearing particles in both micrometric and nanometric scale and the potential ability to induce nucleus- or membrane-oriented damage.

1083 Exposure to Traffic-Generated Air Pollution Increases Systemic and Adipocyte Renin-Angiotensin Pathway Signaling, Lipid Accumulation, and Inflammation in C57Bl/6M Mice Which Is Exacerbated by Consumption of a High-Fat Diet

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Exposure to air pollution is known to contribute to the pathogenesis of cardiovascular disease (CVD). More recent studies have reported a positive correlation between exposure to traffic-generated air pollution and metabolic syndrome and/or obesity in both children and adults. Obesity is strongly correlated with incidence of CVD; however, very limited information exists on the effects of inhaled pollutants on adipocytes or signaling between adipocytes and the cardiovascular system. The renin-angiotensin system (RAS), when dysregulated, is known to mediate pathogenesis in the cardiovascular system, and in adipocytes, through Angiotensin (Ang) II signaling via the Ang II Type 1 (AT1) or Type 2 (AT2) receptor(s). We have previously reported that inhalation exposure to mixed vehicle emissions (MVE) results in increased plasma Ang II, adipocyte size, and adipocyte Angiotensinogen (AGT) and AT-1 expression in C57Bl/6 mice. Thus, we investigated whether MVE-exposure results in altered adipose lipid accumulation and induction of local and vascular inflammatory signaling pathways that contribute to the etiology of obesity and CVD. To test this hypothesis, 3 mo old male C57Bl/6 mice on either a high-fat "Western" diet (HF, 21% fat) or standard (LF, low fat) mouse chow were randomly assigned to inhalation exposure of either filtered-air (FA n=10 per diet) or a mixture of 70 μg PM/m^3 diesel exhaust + 30μg PM/m^3 gasoline exhaust (MVE n=10 per diet) for 6 hr/d for 30 d. MVE exposure resulted in significantly increased adipocyte lipid accumulation (~1.5-fold), as determined by Nile Red staining, which were further exacerbated in MVE+HF diet animals (~5-fold), compared to FA animals. MVE-exposure resulted in significantly increased protein expression at of inflammatory marker, TNF-α, and monocyte/macrophage infiltration (MOMA-2) in both adipose and vascular (aorta) tissue, compared to FA controls. MVE+HF also elevated vascular AT-1 expression that was associated with a 2-fold increase in mRNA expression of vascular adhesion molecular (VCAM)-1, intracellular adhesion molecule (ICAM)-1. These findings indicate that inhalation exposure to traffic-generated pollutants can promote induction of systemic and adipocyte Ang II signaling, lipid accumulation, macrophage recruitment, and inflammation, which are further exacerbated by concurrent consumption of a high fat diet. *Funded by NIH 1R15ES026795 to AKL.*

1084 Exposure to Volatile Organic Compounds Increases Circulating Platelet and Endothelial Cell Microparticles

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Volatile organic compounds (VOCs) are toxicants abundant in both outdoor and indoor air. VOCs are also present in large quantities at several Superfund and Hazardous Waste Sites. Sources of VOCs include burning of fossil fuels or tobacco products and the use of many household commodities like solvents, paints, and glues. While contribution of VOCs in pulmonary disease and cancer is well-studied, little is known about their effect on cardiovascular disease. We examined the effect of VOC exposure on the abundance of circulatory endothelial microparticles, platelet microparticles, and lung microparticles as biomarkers of cardiovascular and pulmonary injury because they have been shown to be positively associated with vascular injury, thrombosis, and respiratory disease. Generalized linear models were used to measure the associations between 16 urinary VOC metabolites and 7 different subtypes of circulating microparticles (measured by flow cytometry) in peripheral blood of 240 non-smokers. Models were adjusted for age, sex, race, BMI, diabetes, and socioeconomic status. Our data show that metabolites of acrolein, acrylamide, 1,3-butadiene, ethylbenzene, and styrene were positively associated with all the seven types of microparticles – endothelial (<1μm/AnnexinV$^+$/CD144$^+$), activated endothelial (<1μm/AnnexinV$^+$/CD144$^+$/CD62E$^+$), endothelial progenitor cell (<1μm/AnnexinV$^+$/CD144$^+$/CD34$^+$), lung (<1μm/AnnexinV$^+$/CD143$^+$), lung endothelial (<1μm/AnnexinV$^+$/CD143$^+$/CD144$^+$), activated lung endothelial (<1μm/AnnexinV$^+$/CD143$^+$/CD144$^+$/CD62E$^+$) and platelet (<1μm/AnnexinV$^+$/CD41$^+$) ($P<0.05$). These microparticles increased by 20-89% per 2-fold increase in VOC metabolite level. Exposure to crotonaldehyde, nn-dimethylformamide, propylene oxide, and o-xylene did not affect platelet microparticles but significantly ($P<0.05$) increased all other microparticles by 26-98% per 2-fold increase in VOC metabolite level. Metabolites of benzene and styrene also increased several types of microparticles by 15-52% per 2-fold increase in metabolite level ($P<0.05$). Together, these data suggest that VOC exposure causes injury to endothelial cells, platelets, and lungs.

1085 The Relationship between Hypoxia and Diesel Exhaust Particle Toxicity and the Effect of a Novel Oxygenating Therapeutic

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Hypoxia plays an important role in the initiation and exacerbation of several adverse health outcomes, including organ transplant failure, insulin-resistance, chronic wound onset, and traumatic brain injury. The influence of hypoxia on disease outcome is directly linked to the accumulation of hypoxia induced factor (HIF) in low oxygen tissues. This transcription factor induces metabolic changes within cells, and is also intertwined with key inflammatory pathways, thereby contributing to the exacerbation of inflammatory responses during periods of cellular stress. This link to inflammation poses a question as to the effect of hypoxia on the toxicity of certain compounds, due to the established link between cytotoxicity and activation of inflammatory pathways. This study aims to understand this link by examining the effect of low-oxygen conditions on diesel exhaust particles (DEP) cytotoxicity in two human and two rat cell-lines. Furthermore, the effect of a novel oxygenating therapeutic, Ox66™, on the relationship between hypoxia and DEP toxicity will be investigated. To assess these relationships, cells were dosed with DEP, Ox66™, and a mixture of both, and then incubated for 24 and 48 hours in both normoxic and hypoxic (~ 2% Oxygen) conditions. After exposure, toxicity was assessed via lactic acid dehydrogenase (LDH) and tetrazolium dye (MTT) colorimetric assays measuring cell damage and viability, respectively. Proteomic analysis was also carried out to measure the levels of interleukin 6, interleukin 8, and tumor necrosis factor alpha present in cell media following exposure. The results reveal a complex relationship between hypoxia and DEP toxicity that is dependent on both cell-type and exposure-time. Additionally, Ox66™ appears to display antagonistic toxicity with DEP in mixtures in some cell-lines, a trend that is exacerbated in hypoxia, pointing to the possible effect of Ox66™ on the amelioration of the adverse effects of hypoxia on cells.

1086 Exposure to Volatile Organic Compounds Depletes Circulating Angiogenic Cells

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Sponsor: D. Hein

Volatile organic compounds (VOCs) are organic chemicals present in both outdoor and indoor air. VOCs are also abundant at various Superfund and hazardous waste Sites. Emerging data suggest that VOCs are associated with many adverse health outcomes, including cardiovascular disease. Endothelial cells line the blood vessel and are critical regulators of blood pressure and vascular function. We hypothesized that VOC exposure prolongs endothelial injury by depleting circulating angiogenic cells (CACs) which repair the damaged endothelium. Generalized linear models were used to measure the associations between 16 urinary VOC metabolites and 15 different subtypes of circulating CACs (by flow cytometry) in peripheral blood of 240 non-smokers. Models were adjusted for age, sex, race, BMI, diabetes status, and socioeconomic status. We observed that metabolites of acrolein, acrylamide, acrylonitrile, 1,3-butadiene, crotonaldehyde, nn-dimethylformamide, ethylbenzene, styrene, and toluene were negatively associated with CAC-6 (CD34+/146+; p<0.05-0.000). CAC6 levels were decreased by 12-29% per 2-fold increase in VOC metabolite level. Exposure to acrylamide, acrylonitrile, nn-dimethylformamide, ethylbenzene and styrene also significantly (P<0.05) depleted CAC-4 (45+/31+/34+/133+) and CAC-14 (45+/34+/133+) by 12-47% per 2-fold increase in VOC metabolite level. Several VOCs also depleted the levels of various other CACs to varying degrees. On the contrary, a metabolite of benzene was positively associated CAC-2, CAC4, CAC-10, and CAC-15--12-47% increase per 2-fold increase in VOC metabolite level. Association of xylene metabolites with CACs was quite complex. While o-xylene metabolite was negatively associated with several CACs, an m-and p-xylene mixture showed a positive association with numerous CACs. These findings suggest that by and large VOC exposure depletes CACs and thereby compromise endothelial repair; however, benzene and m- and p-xylene could increase CACs. Further studies are required to understand this paradox.

1087 Inhalation Exposure to Traffic-Generated Air Pollution Results in Renin-Angiotensin System Deregulation in the Kidneys of ApoE -/- Mice

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Cardiovascular disease (CVD) is the leading cause of death in the world. Epidemiological studies have suggested a strong association between air pollution and the exacerbation of CVD. The renin-angiotensin system (RAS) is known to contribute to CVD, typically through increased expression of one or more RAS pathway components, including renin, angiotensinogen, angiotensin (Ang) II, or signaling through the Ang II receptor type 1 (AT1). Our preliminary data suggest that inhalation exposure to traffic-generated air pollution may upregulate systemic RAS. Thus, we analyzed whether exposure to vehicle emissions (MVE) mediates altered RAS signaling in the kidney. To do this, 6-8 week old male Apolipoprotein null mice ($ApoE^{-/-}$), were fed a high fat "Western" diet for 30 d to promote atherosclerosis. Mice were then randomly assigned to be exposed to either filtered air (FA) or mixed gasoline/diesel engine emissions (MVE: 200 µg PM/m^3 diesel and gasoline exhaust) for 6 hrs/d, 7 d/wk, for 30 d. To investigate the contributing mechanism of RAS-signaling, a subset of control and exposure mice were assigned to receive either an angiotensin-converting enzyme inhibitor (ACEi), Captopril (4mg/kg/day) in their drinking water or drinking water only (vehicle). MVE-exposure resulted in a 3-fold increase in plasma Ang II, compared to FA controls, which was reduced with ACEi treatment, as measured by ELISA. Compared to FA/vehicle controls, expression of inflammatory mediator tumor necrosis factor (TNF)-α mRNA (2-fold induction) and fibrosis (~1.5-fold higher) were also significantly increased in the kidneys of MVE-exposed $ApoE^{-/-}$mice, as determined by RT-qPCR and Masons Trichrome staining, respectively, which were attenuated with ACEi-treatment. Renal AT-1 mRNA levels were observed to be elevated with MVE exposure; however, they were significantly increased in the MVE (2-fold) and FA-ACEi-treated animals, as were renin levels quantified by immunofluorescence. Renin levels were observed to be highest in the MVE+ACEi mice. The increase in ACEi-mediated induction of AT1 and renin is likely due to a feedback mechanism because Ang II plasma levels are decreased through treatment; however, the RAS appears to be deregulated with MVE exposure in these animals leading to significantly increased levels of renin (3-fold increase in MVE+ACEi vs. FA+ACEi). These findings indicate that MVE exposure results in an increase in renal inflammation/fibrosis, and deregulation of the RAS in the kidney, which may contribute to the pathogenesis of CVD.

1088 The Effects of Inhaled Diesel Exhaust Particulate Matter and Probiotic Treatment on Intestinal Microbiota Profiles and Mucosal Integrity in the Ileum of C57Bl/6 Mice

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Recent studies have shown a correlation between air pollution particular matter-exposures and gastrointestinal disorders, which is often associated with alterations in gut microbiota profiles. Alterations in gut microbiota can cause detrimental effects on the mucosal barrier, which not only regulates nutrient absorption but also serves as a physical barrier in defense against pathogens and inflammation. Probiotics have shown promising results in the treatment of gastrointestinal disorders by promoting a healthy and diverse gut microflora. We hypothesize that inhalation of diesel exhaust particles (DEP) will result in changes in gut microbiota and epithelial barrier integrity, which can be mitigated using probiotics in C57Bl/6 mice. Male 4 wk old C57Bl/6 mice fed a high-fat (45%) diet were randomly exposed via oropharyngeal aspiration to 35µg diesel exhaust particles (DEP) suspended in 0.9% saline or saline control twice a week for four weeks. Mice from each exposure were grouped to receive ~0.6g Winclove Ecologic® Barrier 849 probiotic (PRO) treatment or placebo (PLC) via drinking water daily throughout the exposure. qRT-PCR was performed to identify 16s rRNA genes of major bacterial phyla in the ileum. Alcian Blue-Periodic Acid Schiff (AB-PAS) and Hematoxylin and Eosin (H&E) stain were used to determine goblet cells, mucin, and morphology of ileum. Immunofluorescence was performed to determine matrix metalloproteinase-9 (MMP-9) protein expression in ileal tissue. DEP exposure was shown to significantly decrease in *Eubacteria*, *Bacteriodetes*, and *Firmicutes* compared to controls, which was not normalized by PRO-treatment. Preliminary data suggest that DEP-exposure results in reduced goblet cell count and mucin production, and also decreased villi length in the ileum, compared to the controls, which was attenuated with PRO-treatment. Furthermore, MMP-9 is increased in the ileum of DEP- exposed mice, compared to controls, but reduced with PRO-treatment. These findings suggest that exposure to DEP causes significant alterations in microbiota, as well as alterations in the mucosal layer and villi structures, thereby likely contributing to gut dysbiosis. Probiotics effects on MMP-9 expression and villi structure in the DEP-exposed mice suggest microbial changes can influence the effects of environmental particulate matter-exposure on the gut.

1089 Atherogenic Effects of Benzene

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Benzene is a ubiquitous environmental chemical and is abundant at multiple Superfund and hazardous waste sites. In occupational settings, exposure to benzene can reach several hundred ppm. It is also present at in copious amounts in automobile exhausts and cigarette smoke. Multiple studies have shown that benzene, induces hematotoxicity. Recent studies by our group and others have shown that exposure to benzene could induce insulin resistance. However, little is known about the cardiovascular toxicity of benzene. Our ongoing population-based studies suggest that urinary metabolites of benzene such as t,t-muconic acid are associated with increased levels of endothelial microparticles and decreased levels of endothelial progenitor cells, suggesting that exposure to benzene may cause endothelial injury and impair the endothelial repair capacity. Our pre-clinical studies show that a six-week exposure to benzene (50 ppm; 6h/day, 5days/week) in 12 week old male C57BL/6 mice increased the circulating levels of endothelial microparticles (60%, P<0,05), lung endothelial microparticles (61%, P<0.05), and activated endothelial microparticles (64%, P<0.05); and increased platelet-leukocyte adducts in the blood by 1.5-fold (P<0.05) as compared with HEPA-filtered air-exposed mice. Twenty-weeks of exposure to benzene (10 ppm; 6h/day, 5days/week) in 8 weeks old male LDL receptor-knockout mice maintained on western diet increased lesion formation in the aortic valves by 1.4-fold (P<0.05) without affecting plasma lipoproteins. Zymography of aortic valves from showed significantly higher gelatinase activity (activation of matrix metalloproteinases; P<0.05) in lesional macrophages of benzene-exposed mice as compared with the air-exposed mice, suggesting that exposure to VOCs destabilizes atherosclerotic plaques. *In vitro* studies suggest that benzene metabolites benzoquinone, hydroxyquinone, catechol, and phenol induce macrophage apoptosis (cleavage of caspase-3), a critical feature of lesion progression and instability. Collectively, these data suggest that benzene-induces endothelial injury, macrophage apoptosis, exacerbation of atherogenesis and thrombosis, which may increase the risk for myocardial ischemia and stroke.

1090 Suppression of cGAS-STinG Pathway or Inhibition of Mitochondrial NOX-2 Reduces Organic Dust-Induced Mitochondrial DNA-Driven Inflammation in Microglia

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Organic dust (OD) exposure in animal production industries poses serious respiratory and other health risks. OD consists of microbial products and particulate matter. OD exposure induced respiratory inflammation is under intense investigation. However, effect of OD exposure on brain largely remains unknown. Recently, we have shown that OD exposure of brain microglial cells induces an inflammatory phenotype with release of mitochondrial DNA (mtDNA). Therefore, we tested a hypothesis that OD-exposure induced secreted mtDNA signaling drives the inflammation. OD samples were collected from commercial swine operations and a filter sterilized OD extract (ODE) was prepared. Mouse (C57BL/6) microglial cell line were treated with medium or ODE (5%) for 48 hours along with either PBS or mitoapocynin (MA, 10 μM, NOX-2 inhibitor). Microglia treated with control or anti-STING siRNA were exposed to medium or ODE. Next, mouse (C57BL/6) pups were euthanized under an approved protocol, organotypic brain slice cultures (BSCs) were prepared and exposed to medium or ODE with or without MA treatment daily for five days. Culture supernatant, cell pellets and mt-free cytosolic fraction were processed to quantify mt-superoxide, mtDNA, cytochrome C, TFAM, mitochondrial stress markers and mtDNA induced signaling via cGAS-STING and TLR9. Data was analyzed using one-way ANOVA and post-hoc tests. A p value of ≤ 0.05 was considered significant. ODE exposure increased the mt-superoxide formation. Next, MA treatment decreased ODE-induced mtDNA release into cytosol. ODE exposure increased the cytochrome C and TFAM levels. ODE increased MFN1/2 and PINK1 but not DRP1 and MA treatment decreased MFN2. MA treatment decreased the ODE-exposure induced mtDNA signaling via cGAS-STING and TLR9. Anti-STING siRNA decreased the ODE-induced increase in IRF3, IFN-β and Iba1 expression. In BSCs, MA-treatment decreased the ODE induced TNF-α, IL-6 and MFN1. Taken together, OD exposure induced mtDNA signaling could be curtailed through mitochondrial NOX-2 inhibition or STING suppression to reduce neuroinflammation. *In part supported by NIH grants ES026892 and ES027245 to AGK and Iowa State University funding to CC.*

1091 Comparison of Secondary Organic Aerosols from Dark Ozonolysis and OH-Initiated Oxidation in Generating Reactive Oxygen Species

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Secondary organic aerosols (SOA) account for a major fraction of ambient particulate matter, which have significant impact on global climate, air quality and public health. However, the health effects of SOA are still not well understood. The adverse health effects of ambient aerosols are linked to the oxidative potential, known as the capability to generate reactive oxygen species (ROS) *in vivo*. Various acellular assays have been applied for the characterization of oxidative potential, among which electron paramagnetic resonance (EPR) spectroscopy coupled with spin trapping techniques has drawn increasing attention due to its ability of simultaneous quantification of multiple radical species. For lab-generated SOA, OH-initiated photooxidation of precursors, especially biogenic volatile organic compounds (VOCs) has been less investigated in comparison to ozonolysis, especially for health-related studies. Therefore, using EPR spectroscopy, we characterized oxidative potential and radical composition of water reacting with SOA, which were generated from both ozonolysis and OH oxidation. Our preliminary results have shown that SOA from different oxidation systems may introduce highly different radical compositions. To enhance the mechanistic understanding, a kinetic modeling will be utilized to simulate SOA formation and subsequently radical generation from different oxidation pathways. We anticipate that the comparison between different oxidation systems may provide insights into difference in chemical compositions and radical formation mechanisms from associated SOA.

1092 Toxicity Screening of Volatile TSCA Chemicals Using a Novel Air-Liquid Interface *In Vitro* Exposure System

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Recent amendments to the Toxic Substances Control Act (TSCA) include specific provisions that mandate the US Environmental Protection Agency (EPA) to encourage and facilitate "the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions." Though *in vitro* systems have been employed using submerged cultures, these methods do not allow for the testing of volatile/insoluble organic compounds (VOCs), nor do they effectively mimic the physiology of an airway exposure. Thus, a specialized cell culture exposure system (CCES) was developed that permits cells to be exposed at an air-liquid interface (ALI), allowing for direct chemical-to-cell interaction between aerosol, vapor or gas, presented directly to the cells at a constant flow rate and concentration. This novel system is capable of testing six different chemical concentrations simultaneously to generate concentration dependent response curves on a single plate of cells. In the on-going study, BEAS-2B and 16HBE cell lines, as well as primary normal human bronchial epithelial (NHBE) and MatTek Epi-Airway™ cells are used to assess their comparative responses to a variety of volatile chemicals: 1-bromopropane, carbon tetrachloride, dichloromethane, naphthalene, and 1,3-dichloropropene. Cells are exposed for two hours to six concentrations in half-log dilutions, plus an air (sham) control to generate concentration dependent response curves. Cell viability and cytotoxicity are measured via the CellTiter-Glo assay and lactate dehydrogenase release. Our highest doses per chemical induce < 20% cytotoxicity in the BEAS-2B and 16HBE cell lines, while our lowest doses are targeted for no observed adverse effects. Cell lysates have also been collected for whole transcriptome targeted RNA-Sequencing (i.e., BioSpyder TempO-Seq™) and compared for dose response across cell types. Additionally, trans-epithelial electrical resistance (TEER) and ELISA for IL-8 and IL-6 have been measured in naphthalene and 1,3-dichloropropene exposed cells. All endpoints are collected four hours post-exposure. Overall, the main objective of this study is to evaluate the capability of the transcriptomic data to identify concentration-dependent changes in gene expression for volatile chemicals and to evaluate the ability of the transcriptomic data to group chemicals with similar bioactivity profiles for potential read across applications. *Abstract does not reflect views or policies of the US EPA.*

1093 Combinatory Effects of Various Stress Modalities and Acute Ozone Exposure on Stress-Related Gene Expression in the Hippocampus

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Underserved and vulnerable communities are often disproportionally impacted by psychosocial stressors and poor living conditions. These communities are more likely to live in housing near contaminated sites and/or closer to busy roadways. Epidemiological studies have shown that children from disadvantaged communities exposed to various environmental contaminants have increased incidence of asthma and obesity, both morbidities that are also related to increased chronic stress. This study sought to identify how community-level stressors (noise, confinement, fear, and other life stressors) can interact with air pollutants to increase susceptibility to stress. A rat model of chronic stress was developed by exposing rats to unpredicted stressors (restraint, Tilted cage, Shaking, Noise, Predator odor) for a period of 2 months and then tested for its susceptibility to ozone-induced (environmental stressor) gene expression changes in the brain region involved in stress adaption (hippocampus). Another set of rats were single housed during this 8-week period, with no environmental enrichment provide, to model "psychosocial" stress. Rats were exposed to filtered air or ozone (0.8 ppm) for 4h followed immediately by necropsy. We designed a qPCR primer-based array for genes related to glucocorticoid, GABAergic, glutamatergic, and adrenergic signaling as well as stress, neurotrophics and the endocannabinoid system. We found that ozone decreased expression of glucocorticoid receptor genes (*nr3c1*, *nr3c2*) independent of stressor. Both stress groups displayed apparent decreases in *fkbp4*, with no effect of ozone. One of the most profound gene expression changes was found with *fkbp5*, where ozone dramatically increased expression independent of stress group. Ozone increased gene expression for *avp*. We found increased expression of *Crhr2* only in the no

stress group, this effect was blocked in all stress groups. Additionally, ozone decreased expression of *bdnf* across all groups. These results demonstrate a glucocorticoid mediated signal (*nr3c1*, *nr3c2*, *fkbp4*, *fkbp5*) in the hippocampus following ozone exposure. *This abstract does not necessarily reflect US EPA policy.*

PS 1094 ERV1/ChemR23 Protects against Ozone-Induced Pulmonary Inflammation and Injury

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Inhalation of ground level ozone (O_3) poses several potential health risks for the global population. Numerus epidemiological studies show that ozone exposure can lead to cardiovascular health problems, aggravate lung diseases such as asthma, and cause damage to the lungs. Ozone is known to induce pulmonary inflammation and injury, but the mechanisms of this damage are poorly understood. In our previous studies, 24 hours post ozone exposure was found to have significant reductions in pulmonary specialized pro-resolving lipid mediators (SPMs). SPMs are involved in the resolution of inflammation and are a broad family of lipid mediators, including resolvins, protectins, and maresins that are derived from eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA). SPMs regulate pro-resolving pathways through G-protein coupled receptors, such as chemokine-like receptor 1 (ERV1/ChemR23). ChemR23 is a receptor for chemerin, a chemoattractant for macrophages, and EPA-derived SPM resolvin E1. Currently, the role of SPM signaling through these G-protein coupled receptors following ozone-induced pulmonary inflammation and injury is unknown, and the goal of this study is to explore ChemR23 and SPM signaling in this context. Female wild type (ChemR23$^{+/+}$) or ChemR23 deficient (ChemR23$^{-/-}$) mice 8-10 weeks of age were exposed to 1ppm ozone for 3 hours and necropsied 24 hours post-exposure. Bronchoalveolar lavage (BAL) was obtained to measure pulmonary inflammation and injury. Total protein in the BAL was measured as a marker of pulmonary microvascular injury and BAL cellular differentials were quantified to determine cellular infiltrates after exposure. 24 hours post ozone exposure, ChemR23$^{-/-}$ mice showed significantly increased airspace neutrophilia and pulmonary injury via higher BAL protein levels compared to the ChemR23$^{+/+}$ controls. These data suggest that the EPA-derived resolvin E1 receptor ChemR23 plays a significant role in the resolution of ozone-induced pulmonary inflammation and injury. Future studies will uncover if these findings correlate with decreased SPM production 24 hours after ozone exposure, and if a diet supplemented with RvE1 or its parent compound mitigates ozone-induced pulmonary inflammation and injury.

PS 1095 Long-Term Airway Exposure to Environmental Allergens Increases Blood Pressure and Myocardial Vulnerability and Alters Responsiveness to Ozone

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Asthma and allergic rhinitis result from the interplay of genetic determinants and exposure to inhaled environmental triggers. While the respiratory impacts of allergic airways disease have been well-studied, the burden on the cardiovascular system has received comparatively little attention. Importantly, epidemiological studies have demonstrated that asthma increases risk for stroke and coronary heart disease. The precise cardiovascular risks associated with allergic airways disease and the biological mechanisms responsible, however, remain unknown. The purpose of this study was to, for the first time, determine the impacts of allergic airways disease on cardiovascular function in an experimental model. To model allergic airways disease, female rats were intranasally instilled for 6 weeks with a mixture of relevant environmental allergens (i.e. house dust mite, aspergillus fumigatus, and ragweed) previously shown to reliably elicit chronic features of asthma. At the conclusion of the allergen regimen, rats were exposed once to 0.5 ppm ozone to assess cardiovascular sensitivity to a prototypical air pollutant. Cardiovascular function, including blood pressure and the electrocardiogram, was constantly monitored using implantable telemetry. Sensitivity to the cardiac arrhythmogenic agent aconitine was also assessed. Preliminary data indicates that pulmonary impacts were characteristic of allergic airways disease. Moreover, long-term exposure to allergens increased diastolic blood pressure ($p<0.05$) and to a lesser extent systolic blood pressure ($p=0.06$) and increased sensitivity to aconitine-induced cardiac arrhythmia ($p<0.05$). Furthermore, ozone decreased heart rate to a similar extent ($p<0.05$) in both saline and allergen-instilled groups and increased sensitivity to aconitine only in the saline-instilled group ($p<0.05$). These findings demonstrate that allergic airways disease may increase cardiovascular risk in part by altering blood pressure and by causing cardiac electrical instability. *This abstract does not reflect US EPA policy.*

PS 1096 Glucocorticoid and Beta-Adrenergic Receptor Antagonists Inhibit O_3-Induced Metabolic Response

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Inhalation of air pollutants has been linked to neuroendocrine stress-induced systemic metabolic and innate immune effects through increased circulating adrenaline and glucocorticoids. We have shown that ozone (O_3) induced pulmonary injury and inflammation are mediated through the activation of beta-adrenergic receptors (BAR) and glucocorticoid receptors (GR). Since air pollution has been linked to increased incidence of diabetes and obesity, assessing the contribution of stress mediators in metabolic homeostasis can aid in understanding the mechanisms. The purpose of this study was to examine if BAR and GR are involved in O_3-induced systemic and liver metabolic alterations. To determine the impact of BAR and GR individually or in combination, we treated 11-13-week-old male Wistar-Kyoto rats with propranolol (PROP), a non-specific BAR antagonist, and mifepristone (MIFE), a GR antagonist. Treatments began 7 days prior to air or O_3 (0.8 ppm x 4 h) exposure and ended the day of exposure. Animals received injections of 1) saline or PROP (10 mg/kg/day, i.p.); 2) corn oil or MIFE (30 mg/kg/day, s.c.) or 3) both PROP+MIFE. O_3-induced hyperglycemia was mitigated by MIFE and PROP+MIFE but not PROP alone, however, both drugs reduced O_3-induced glucose intolerance. Circulating cholesterol (CHOL) and free fatty acids (FFA) increased after O_3 exposure as we have observed in other studies. This effect on CHOL was mitigated by MIFE and PROP+MIFE, but not PROP. O_3 effect on FFA was mitigated by MIFE and/or PROP. Liver expression of genes involved in glucose and lipid metabolic processes showed O_3-induced increases in glucose 6-phosphatase (G6pc) and hexokinase-2. The effect on G6pc was mitigated by MIFE suggesting a role of GR in O_3-increased gluconeogenesis. Neither PROP nor MIFE had any effect on O_3-induced increases in carnitine palmitoyl transferase 2, peroxisome proliferator activated receptor alpha and gamma, and decreased steroid responsive element binding factor 2. In conclusion, while GR activation played a major role in O_3-induced glucose and lipid metabolic changes, BAR inactivation was associated with mitigation of peripheral glucose uptake. Thus, O_3-induced homeostatic metabolic changes support epidemiological observations, and could contribute to metabolic syndrome in susceptible individuals. *This abstract does not reflect the US EPA policy.*

PS 1097 Ozone Inhalation Impacts Placental Biomarkers and Uterine Artery Function

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Adverse environmental stressors in the early stages of life can increase the risk of developing chronic diseases and may play a significant role in cardiovascular disease in adulthood. Respectively, air pollution exposure has become an area of interest and is being recognized as an important risk factor associated with adverse pregnancy outcomes. Recently, the placenta, as a target organ for maternally-inhaled pollutants has gained recognition as a potential mediator of adverse birth outcomes related to ambient air pollution, such as ozone. The present study examined the mechanism(s) by which inhaled pollutants may contribute to adverse cardiovascular conditions. We investigated effects on the uterine artery in this context, by reviewing its pathology and how particulate air pollution has a toxic effect on development and the causes of adverse pregnancy outcomes. We evaluated both inflammatory and oxidative stress markers following maternal ozone exposures. We began this project by evaluating how serum from ozone exposed rats caused uterine artery contractility and compared the results to serum from filtered air exposures. We determined that serum from ozone-exposed rats caused a ~20% net increase in uterine artery contractility when compared to serum from filtered air exposure. *In vivo*, this was seen as increased uterine artery resistance index, as assessed by ultrasonography. During preliminary studies filtered air (FA)-exposed rats also showed enhanced uterine artery vasodilation when treated with acetylcholine as compared to 0.3ppm and 1ppm ozone exposed rats. We further analyzed placental gene expression by quantitative polymerase chain reaction (qPCR). Our genes of interest included: chemokine

ligand 1 (CXCL-1), chemokine ligand 2 (CCL2), collagen type 1 alpha 1 chain (COL1A1), transforming growth factor beta 1 (TGFB1), frizzled class receptor 1 (FZD1), cyclooxygenase 1 (COX1), cyclooxygenase 2 (COX2), chemokine ligand 2 (CCL2), and matrix metalloproteinase 10 (MMP10). Patterns of key transcript changes (MMP10, Col1a1, and FZD1) aligned with human mRNA changes in preeclampsia, supporting the potential that early (gestational day 10) exposures could promote or exacerbate gestational hypertension later in pregnancy.

1098 CD163 Protects against O_3-Induced Oxidative Stress and Pulmonary Injury

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Epidemiological studies have reported that exposure to elevated ambient concentrations of ozone (O_3) is associated with adverse pulmonary and cardiovascular health outcomes. However, the underling biological mechanisms mediating O_3 associated adverse health effects are unknown. O_3 induces oxidative stress in the lung leading to pulmonary inflammation and injury. This pulmonary inflammation and injury correlates with increased levels of damage associated molecular patterns (DAMP). Recently we have found that O_3 exposure is associated with increased levels of the DAMP cell free hemoglobin (CFH). CFH is endocytosed by CD163, a scavenger receptor exclusively expressed on macrophages/monocytes; the uptake results in the up-regulation of heme oxygenase 1 (HO-1). Therefore, we hypothesize that O_3 -induced oxidative stress increases CFH in the airspace and that CD163 is critical for protecting against this DAMP induced injury. Methods: C57BL/6J (WT) and CD163$^{-/-}$ male mice were injected with vehicle or 100 mg/kg of N-Acetyl-Cysteine (NAC) and then, 1h later, exposed to either filtered air (FA) or 1ppm O_3 for 3h. BAL and blood samples were obtained 6h or 24hr post exposure and bronchoalveolar lavage (BAL) cell counts, CFH, and total protein were measured. Real time PCR was used to measure HO-1, proinflammatory cytokine, and chemokine levels in lung tissue. Results: After O_3 exposure, WT mice had a significant increase in CFH in the BAL which was augmented in O_3 exposed CD163$^{-/-}$ mice. Additionally, O_3 exposed CD163$^{-/-}$ mice had greater pulmonary inflammation and injury compared to WT mice as evidenced by increased BAL neutrophils, macrophages, and total protein. CD163$^{-/-}$ mice exposed to O_3 also had significantly lower levels of HO-1 when compared to WT controls. In WT mice, NAC pretreatment did not alter O_3-induced pulmonary inflammation or injury whereas NAC pretreatment of CD163$^{-/-}$ mice significantly decreased BAL protein. Our findings suggest that the mitigation of O_3-induced oxidative stress is dependent on CD163 due to its ability to clear excessive DAMPs such as CFH. Future studies will examine the underlying mechanism of CFH-augmented oxidative stress and its relationship to monocyte/macrophage efferocytosis.

1099 Exacerbation of Sepsis-Induced Acute Lung Injury (ALI) by Ozone Is Regulated by Macrophage Phenotypic Switching

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Ozone (O_3) is an urban air pollutant known to cause alveolar epithelial barrier damage and altered pulmonary functioning. EPA compliant levels of O_3 are linked to an increased incidence of acute respiratory distress syndrome, a severe form of acute lung injury (ALI). We previously showed that O_3 exacerbates sepsis-induced ALI by promoting neutrophil accumulation in the lung. In these studies, we analyzed the role of resident and inflammatory macrophages in this pathogenic response. Male C57Bl/6J mice were exposed to O_3 (0.8 ppm) or air for 3 h followed 24 h later by i.v. lipopolysaccharide (LPS) (2.5 mg/kg) to model bacterial sepsis or PBS control. Bronchoalveolar lavage (BAL) was collected 24 and 48 h later, and fluid analyzed for markers of lung damage (protein, IgM, phospholipids). BAL cells were magnetically separated based on Cd11b expression and subsets characterized by flow cytometry as resident alveolar macrophages (CD11b$^-$CD45$^+$SiglecF$^+$Ly6G$^-$CD11c$^+$F4/80$^+$), inflammatory macrophages (Cd11b$^+$CD45$^+$SiglecF$^-$ Ly6G$^-$CD11c$^-$F4/80$^+$) and neutrophils (CD11b$^+$CD45$^+$SiglecF$^-$Ly6G$^+$CD11c$^-$F4/80$^-$). Lung damage was significantly increased in mice exposed to O_3+LPS at 24 h relative to the other groups, a response correlated with increased numbers of neutrophils. At this time, *Nos2, Il1β, Cxcl1, Ccl2,* and *Ptgs2* mRNA expression was upregulated in resident macrophages. By 48 h, lung damage abated and resident macrophage expression of these genes decreased, while *Il4* and *Arg1* expression increased suggesting a switch from an M1 (proinflammatory) to an M2 (anti-inflammatory) phenotype. In O_3+LPS treated mice, infiltrating macrophages increased in the lungs at 24 and 48 h post exposure. Whereas at 24 h, these cells were mainly *Nos2* and *Ptgs2* positive, by 48 h they were *Arg1* positive indicating a similar phenotypic switch from a pro- to an anti-inflammatory phenotype. These results suggest that the O_3 exacerbation of ALI is due to early pro-inflammatory activation of resident and infiltrating macrophages, which release reactive nitrogen species and recruit neutrophils into the lung. Subsequent resolution of injury occurs as a consequence of macrophage phenotypic switching. *Supported by NIH ES004738, ES005022, IRSS Donald E. Gardner Toxicology Education Award.*

1100 Independent Roles of Beta-Adrenergic and Glucocorticoid Receptors in Systemic and Pulmonary Effects of Ozone

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The release of catecholamines is preceded by glucocorticoids during a stress response. We have shown that ozone inhalation produces homeostatic stressor effects through adrenergic receptor (AR) and glucocorticoid receptor (GR) activation by these adrenal-derived stress hormones. In this study, we 1) inhibited beta AR (BAR) while inducing GR or 2) inhibited GR while inducing BAR to examine the role of each receptor without the interactive effects of the other following ozone exposure. The treatment with each antagonist began 7 days prior to exposure to assure complete inhibition of receptors while agonist treatment occurred only 1 day prior. Twelve-week old male Wistar-Kyoto rats were treated daily with saline or propranolol (PROP; a non-selective BAR antagonist, 10 mg/kg, i.p.) starting at day 1 and with saline or dexamethasone sulfate (DEX; GR agonist; 0.02 mg/kg) starting at day 6 and during each day of air or 0.8-ppm ozone exposure (day 7 and day 8; 4h/day for 2 days). In the second experiment, rats were treated with saline or mifepristone (MIFE; GR antagonist, 30 mg/kg, s.c.) starting at day 1 and with saline or clenbuterol (CLEN; BAR agonist; 0.02 mg/kg) starting at day 6 and during each day of the same 2-day air or ozone exposure, followed by necropsy within 2 hours. DEX and PROP+DEX decreased adrenal, spleen and thymus weights in all rats. Ozone increased plasma epinephrine with minimal drug-treatment effect. DEX decreased and MIFE increased corticosterone levels. Ozone-induced increases in lung injury, protein leakage, inflammation and lavage fluid IL-6 were inhibited by PROP and exacerbated by CLEN. DEX and ozone decreased circulating lymphocytes, while MIFE and MIFE+CLEN reversed this effect. DEX exacerbated, while MIFE, PROP or MIFE+CLEN inhibited, ozone-induced hyperglycemia and glucose intolerance. Ozone inhibited glucose-induced insulin release with each drug having variable effect. In summary, 1) activating BAR, even with inhibition of GR, exacerbated ozone-induced pulmonary effects and inhibiting BAR attenuated these effects; and 2) activating GR exacerbated ozone systemic effects, even when BAR was inhibited. These data provide further insights on the independent role of BAR in pulmonary effects and roles of both BAR and GR in systemic metabolic effects of ozone. *Does not reflect the US EPA policy.*

1101 Identification of Candidate Extracellular Vesicle microRNA Regulators of Ozone-Induced Airway Inflammation

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Inhalation exposure to the air pollutant ozone (O_3) is associated with airway inflammation, exacerbation of existing respiratory diseases, and heightened susceptibility to respiratory infections. O_3 rapidly oxidizes components of the airway surface to form biologically active molecules that initiate these adverse effects. The precise mechanisms by which secondary O_3-airway surface liquid reaction products elicit inflammation and impair host defense are not fully known. Extracellular vesicles (EVs) are nano-scale, lipid membrane enclosed particles known to transport microRNAs (miRNAs) that can regulate mRNA expression in recipient cells. We hypothesized that EV-miRNAs regulate the transcriptional program of airway cells (e.g., epithelial cells and macrophages) in a manner that contributes to O_3-induced inflammation. To explore this, we exposed female C57BL/6J mice to filtered air (FA), 1, or 2 ppm O_3 by inhalation and 21 hours later collected bronchoalveolar lavage fluid (BALF) and isolated conducting airways (CA) for markers of airway inflammation, EVs, and airway macrophages (AM) and airway epithelia, key O_3-responsive cell types. We isolated EV-small RNAs and total RNA from AM for RNA sequencing (RNA-seq). O_3 exposure caused significant increases in airway EVs as measured by Nanoparticle Tracking Analysis (NTA) in parallel with traditional

markers of inflammation, such as inflammatory cells (macrophages and neutrophils) and protein in the BALF. Analysis of AM and CA mRNA and EV-miRNA by RNA-seq revealed an abundance of O_3-induced transcriptomic changes, including 693/971 differentially expressed AM mRNAs, 903/2148 differentially expressed CA mRNAs, and 59/149 differentially expressed EV-miRNAs in O_3 exposed mice (1/2 ppm O_3 vs. FA). Several differentially expressed EV-miRNAs in the O_3 groups, including miR-2137, miR-501, and miR-34a, exhibited concentration response patterns of expression. Integrative analysis of EV-miRNA and AM mRNA expression data identified EV-miRNAs 99a-5p and 22a-3p as candidate regulators of AM transcriptomic responses to O_3, targeting several genes including *Igf1r* and *Sp1*, respectively. In contrast to AM, we did not identify putative EV-miRNA regulators of CA gene expression through our bioinformatic analyses. In summary, our data show that O_3 exposure alters AM and CA gene expression, EV release, and EV-miRNA content, suggesting further investigation of EVs – specifically how they affect AM function - may provide insight into the mechanisms of O_3-induced respiratory inflammation and impaired host defense.

1102 Exosomal microRNA Expression Is Associated with Ambient Ozone Exposure in Coronary Artery Disease Patients

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Exposure to ambient air pollutants such as ozone (O_3) and particulate matter (PM) is associated with increased cardiovascular morbidity and mortality, but the underlying biological mechanisms have yet to be described. Emerging evidence shows that gene regulation by microRNAs (miRNAs) may play a role in air pollution-induced cardiovascular risk. This study aims to explore the association between exposure to ambient air pollutants and exosomal miRNA changes related cardiovascular disease. Fourteen participants with coronary artery disease were enrolled in this panel study. Each participant had up to 10 visits and their plasma samples were collected and measured for expression levels of exosomal miRNA 21 (miR21), miR126, miR146, miR150, and miR155 using quantitative RT-PCR. Daily 24-hr measurements of O_3 and $PM_{2.5}$ were obtained from central monitoring stations up to 4 days prior. Mixed effects models adjusted for temperature, humidity, and medications were used to assess the association between miRNA changes and ambient air pollutant levels. Expression of miR21 was negatively associated with ambient O_3 levels with a 3-day lag while miR150 expression was positively associated with a 1-day lag and 3d- and 5d- moving average ($P<0.05$). miRNA changes were not significantly associated with ambient $PM_{2.5}$ levels up to 4-day lag time. Further analysis indicated that β-blocker usage significantly modified the association between ozone exposure and exosomal miRNA levels where the association was more prominent among non-users. Conclusions: Exposure to ambient O_3, not $PM_{2.5}$, was associated with changes of exosomal miRNA levels in plasma. Ambient O_3 exposure may cause cardiovascular effects through altering miRNA levels in coronary artery disease patients. *This abstract of a proposed presentation does not necessarily reflect US EPA policy.*

1103 Ozone-Derived Oxysterols Impair Lung Macrophage Phagocytosis by Adduction of Phagocytosis Receptors

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Ground-level ozone is a common air pollutant found in photochemical smog and is known to cause pulmonary inflammation and suppress bacterial clearance in the lung. However the mechanism driving these effects are not fully understood. Ozone reacts with cell-derived cholesterol to form oxysterols, including the highly reactive secosterol A (secoA). SecoA or its aldol condensation product secosterol B (secoB), can adduct to lysine residues resulting in covalent modification of proteins, yet, the significance of secoA/B adduction on cellular function in lung macrophages is not known. We hypothesize that adduction of macrophage proteins by secoA/B results in impaired protein function. Protein adducts from human BAL macrophages were identified using an alkynyl-tagged secoA (20μM). Adducted proteins were identified using "click" chemistry to isolate alkynyl-tagged proteins followed by identification using mass spectrometry. Adduct confirmation was done using western blot. Binding of the phagocytic receptors CD206, CD64, and CD16a was measured via an in-house binding ELISA. The effect of oxysterols on phagocytosis was measured by the uptake of pH sensitive zymosan bioparticles in THP-1 macrophages and uptake of opsonized bacterial bioparticles in BAL macrophages. We identified over 100 proteins that formed adducts with secoA in BAL macrophages, including CD206, a known factor in macrophage phagocytosis and marker of M2 polarization. Treatment of recombinant human CD206 with secoA or secoB resulted in decreased binding to a known CD206 ligand. Similarly, the binding of Fc receptor member CD64 was impaired by secoA. Interestingly, binding of another Fc receptor, CD16a, was unaffected by oxysterols suggesting that not all endocytic receptors are affected by secoA treatment. *In vitro* experiments showed that uptake of both zymosan (mediated through CD206) and opsonized bacteria (mediated through Fc receptors) were impaired with oxysterol treatments. Our work shows that oxysterol adduction of the phagocytic receptor CD206 results in impaired receptor binding to target ligands. This represents a novel mechanism for impairing phagocytosis in ozone exposed macrophages.

1104 Altered Transcriptomic Effects of Ozone across the Brain following Maternal High Fat-Diet

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Obesity affects more than one-third of US adults and is one of the leading causes of preventable death. Previously, we have found that dams fed a high fat diet (HFD) for 6-weeks prior to breeding and through postnatal day (PND) 32, generated offspring with significantly elevated susceptibility to ozone (O_3) when exposed as adults. We concluded that poor maternal diet choices may alter offspring's susceptibility to air pollutants. We hypothesized that maternal HFD would alter the transcriptome of the hypothalamus (HYP) and impact the effect of O_3 on global gene expression in both HYP and hippocampus (HIP). A 6-week pre-breeding HFD treatment was performed alongside a control diet (CD) on Long Evans dams and continued through PND 30 when all offspring were switched to control diet. At PND 40, rats were exposed to filtered air or ozone (0.8 ppm for 5 hr), followed by necropsy. Total isolated RNA (RIN score > 6) was prepped for using a PrepX RNA-Seq kit (Takara) and sequenced on an Illumina NextSeq 500 for 75 single-read cycles. Sequencing data was aligned to rn6 Ensemble Transcripts (r97), outliers identified by principal components analysis, and differentially expressed genes (DEGs) were determined by DEseq2 using a false discovery rate cutoff of <0.05. In the HYP, HFD increased expression of *PCDH20*, which encodes for a non-clustered protocadherin involved in neuronal diseases. O_3 exposure blocked this increase. HFD also increased expression of *ACADSB* in the HYP, which is involved in dietary protein processing. Exposure to O_3 reversed this expression. Within the HYP of CD, O_3 exposure resulted in 17 DEGs, 11 of which were also altered in the HYP of HFD exposed to O_3. In the HIP, the O_3 induced only 7 ozone-mediated DEGs identified in CD. O_3 influenced the expression of the majority of the DEGs. The most consistent effect of ozone across both brain regions was an increased expression of the lipid droplet storage factor, *Plin4*. These results indicate that ozone induces changes in gene expression in both the HYP and HIP and maternal diet may interact with these expressions. *This abstract does not necessarily reflect US EPA policy.*

1105 Susceptibility to Ozone-Induced Metabolic and Immunological Alterations in Rat Models of Social Isolation and Mild Chronic Stress

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Underserved vulnerable communities are disproportionally impacted by psychosocial stressors and poor living conditions. Epidemiological studies have shown an interaction between psychologically stressed individuals and air pollution exposure. Our lab and others have recently demonstrated that ozone exposure activates neuroendocrine stress response pathways leading to systemic metabolic and inflammatory responses. The purpose of this study was to identify whether community level stressors (i.e. noise, uncomfortable living conditions, confinement, fear) can interact with ozone to increase susceptibility for immunological and metabolic diseases. Male, 4-week-old Wistar-Kyoto rats were randomized into 3 stress groups: No Stress (NS) - control group; Social Isolation (SI) - single-housed; Chronic Stress (CS) - single-housed and subjected to mild randomized stressors (i.e. restraint, tilted cage, shaking, intermittent noise, and predator odor) 5 days/week for 8 weeks. Animals were then exposed acutely to filtered air or ozone (0.8 ppm) for 4h followed by necropsy. After 3 weeks on the stress protocol through the end of the study, the CS group had significantly decreased body weight compared to the NS and SI groups. This corresponded with increased adrenal weight and urine corticosterone and metanephrine levels measured in the CS group, suggesting the chronic stress protocol was successful. In air-exposed rats, SI and CS tended to cause depletion of circulating thyroid stimulating hormone (TSH), luteinizing hormone (LH), prolactin, follicle stimulating hormone, and brain-derived natriuretic factor (SI>CS), but not adrenocorticotrophic hormone. SI also in-

duced more severe systemic inflammation than CS, as evidenced by increases in circulating cytokine levels (i.e. IL-1β, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-γ) regardless of exposure. Ozone exposure led to increases in plasma corticosterone levels (NS and SI), plasma epinephrine (CS), and urine corticosterone and catecholamine metabolites (all groups). Exposure to ozone also led to increases in serum glucose, leptin, and branched-chain amino acids; increases in BALF IL-6 and TNF-α; and decreases in serum TSH, prolactin, and LH, with small influence of CS and SI. Systemic inflammation and some pulmonary effects of ozone were exacerbated by SI. Collectively, these data suggest that the degree to which psychosocial stressors affect the neuroendocrine system will likely sway the adverse metabolic and inflammatory responses induced by ozone exposure, a challenge stressor. *Does not reflect the US EPA policy.*

1106 Depletion of CD11b$^+$ Alveolar Macrophages Exacerbates Lung Injury and Oxidative Stress following Ozone Inhalation

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Ozone (O_3) is an urban air pollutant known to cause lung inflammation, injury and oxidative stress. CD11b$^+$ macrophages accumulate in alveolar and interstitial regions of the lung following O_3 exposure and have been characterized as Ly6C^{hi} proinflammatory and Ly6C^{lo} anti-inflammatory/wound repair. To assess the role of CD11b$^+$ macrophages in O_3 toxicity, we used mice with a transgenic diphtheria toxin (DT) receptor under the control of the CD11b promoter (CD11b-DTR). Mice were treated ip with DT (25 μg/kg) to deplete CD11b$^+$ monocytes/macrophages. After 1 h, mice were exposed to air or 0.8 ppm O_3 for 3 h in whole body chambers. Mice were euthanized 24 h later and bronchoalveolar lavage fluid and lung collected. Following exposure of DT-treated CD11b-DTR mice to O_3, numbers of CD11b$^+$ Ly6C^{hi} proinflammatory alveolar macrophages increased relative to non-DT treated mice, while Ly6C^{lo} anti-inflammatory alveolar macrophages decreased. This correlated with increases in BAL protein levels indicating exacerbation of O_3-induced lung injury in DT-treated CD11b-DTR mice. O_3-induced oxidative stress, as reflected by increases in YM-1 expression, also increased in these mice. O_3 exposure also caused an increase in CD11b$^+$ Ly6C^{hi} and Ly6C^{lo} interstitial macrophages in lungs of non-DT treated mice; both inflammatory macrophage subpopulations were significantly reduced after DT administration to the mice. This was associated with decreased expression of inducible nitric oxide synthase in the lung consistent with fewer proinflammatory macrophages migrating into alveolar spaces from the interstitium following O_3 exposure. Conversely, DT administration had no effect on O_3-induced neutrophil accumulation in the lung. These data indicate that inflammatory monocyte-derived macrophages that localize in the alveolar regions of the lung after O_3 exposure are derived from monocyte and interstitial macrophage precursors; moreover, these cells play a key role in dampening O_3-induced oxidative stress and alveolar epithelial barrier dysfunction. Elucidating the role of inflammatory macrophage subpopulations in O_3 toxicity is key to understanding mechanisms of lung injury and developing approaches for mitigating tissue damage. *NIH ES004738, AR055073 ES005022; EUH 778051; MSHEP 3899/H2020/2018/2.*

1107 Cardiac microRNAs Relevant to Inflammation, Endothelial Function, and Myocardial Function: Impact from Ozone Exposure and Dietary Supplementation

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Air pollution exposure has been associated with adverse cardiovascular effects. Fish oil (FO) and olive oil (OO) supplementation attenuates the cardiovascular responses to air pollutants in human subjects. MicroRNAs (miRNAs) are a key regulating factor for gene expression. We examined the influence of ozone (O_3) on miRNAs relevant to inflammation, endothelial function, and cardiac function and the impact of dietary supplementation with FO and OO on O_3-induced changes in miRNAs expression in rat hearts. Male Wistar Kyoto rats were fed either a normal diet (ND), a diet enriched with 6% FO or OO starting at 4 weeks of age. Eight weeks following the start of these diet, animals were exposed to filtered air (FA) or 0.8 ppm O_3, 4 hr/day for 2 consecutive days. Selected miRNAs of inflammation, endothelial and cardiovascular function were assessed in cardiac tissues to examine the effects of diets and O_3 exposure. Cardiac miRNAs were altered by both diet and O_3 exposure. Specifically, O_3 exposure was associated with up-regulation of miR-150 and miR-208a and down-regulation of miR-107 and miR-145 in ND, as well as down-regulation of miR-150 in FO and up-regulation of miR-21, miR-29 and miR-486 in OO. O_3-induced up-regulation of miR-150 and down-regulation of miR-21 were attenuated by both FO and OO. These results suggest that acute O_3 exposure-induced inflammation and cardiac and endothelial dysfunction may be mediated by effects of O_3 on miRNA expression, and dietary supplementation with FO or OO may alleviate these adverse effects in rats. *This abstract of a proposed presentation does not necessarily reflect US EPA policy.*

1108 Systemic Inflammation and Peripheral Organ Injury after Ozone Exposure in Diabetic Rats Receiving Atherogenic Diet

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Exposure to air pollutants is associated with increases in cardiometabolic diseases among susceptible individuals. While we have shown that ozone induces neuroendocrine stress-mediated homeostatic alterations in multiple organs, susceptible individuals with altered stress response may be predisposed to exacerbation of chronic diseases. The purpose was to determine the susceptibility of diabetic rats on control or atherogenic diets to acute ozone-induced systemic and peripheral organ changes in immunological and metabolic processes. Healthy, male Wistar and Wistar-derived Goto-Kakizaki (GK) rats, genetically predisposed to non-obese type 2 diabetes, received control or high-cholesterol atherogenic (HCA) diet beginning at 4 weeks of age for 12 weeks. Following the 12 week diet regimen, rats were exposed to air or 1 ppm ozone, 6h/day for 1 or 2 days and responses were analyzed at the end of each exposure. GK rats on the control diet were predisposed to hyperglycemia and glucose intolerance and increased body fat percentage relative to their Wistar counterparts; however, serum cholesterol and triglycerides levels were comparable between both strains. HCA diet increased circulating cholesterol, low-density lipoprotein, and insulin in both strains regardless of exposure. Increases in insulin levels found in GKs on the HCA diet were exacerbated after ozone exposure. Circulating lipase increased with HCA diet in both strains (Wistar>GK) and exposure groups. Likewise, circulating aspartate and alanine amino transferases increased in both strains receiving HCA diet with ozone exacerbating effects in GK rats, indicating liver injury. Circulating cytokines such as IFN-gamma, TNF-alpha, IL-6, and KC/GRO increased in both strains on HCA diet, and ozone, exacerbated these responses. Gene expression for metabolic markers was assessed using targeted Illumina sequencing in liver and muscle for both strains on control diet. Principle component analysis indicated strain differences in lipid and glucose metabolic markers and marked ozone effects in both liver and muscle, suggesting differential ozone impact in GK rats. These data provide insights into how ozone may exacerbate peripheral metabolic and systemic inflammatory changes in susceptible individuals on an unhealthy lipid-rich diet. *Does not reflect the US EPA policy.*

1109 Valproic Acid Blunts Lung Injury, Oxidative Stress, Inflammation, and Altered Pulmonary Mechanics Induced by Inhaled Ozone

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Ozone is an air pollutant known to cause oxidative stress and inflammation in the lung. This can contribute to lung injury and aberrant pulmonary functioning. In the present studies we assessed the effects of valproic acid (VPA), a histone deacetylase inhibitor with antioxidant and antiinflammatory activity, on ozone-induced pulmonary toxicity. Female C57B16/J mice (18-22 g) were exposed to air or ozone (0.8 ppm, 3 h) in whole body chambers. This was followed 0.5 and 24 h later by i.p. administration of PBS control or VPA (300 mg/kg). Mice were euthanized 48 h later and bronchoalveolar lavage (BAL) and tissue collected. Ozone exposure resulted in increased levels of protein, surfactant protein-D (SP-D), IgM and cells in BAL, indicative of lung injury and inflammation. Ozone also caused oxidative stress as measured by disruption of SP-D structure, and increases in lung heme oxygenase-1 (HO-1) and 4-hydroxynonenal (4HNE)-modified proteins. Treatment of mice with VPA significantly reduced ozone-induced increases in BAL SP-D levels and blunted alterations in SP-D structure; HO-1 and 4HNE modified protein expression was also decreased. Flow cytometric analysis of BAL lung cells showed that ozoneinduced injury and oxidative stress were associated with increases in proinflammatory macrophages in the lung. These cells expressed ARL11 and TNFα, demonstrating that they are activated. VPA treatment reduced the number of activated macrophages accumulating in the lung in response to

ozone; VPA also suppressed the accumulation of monocytic and granulocytic myeloid-derived suppressor cells (MDSC). Ozone-exposure caused alterations in pulmonary function, including increases in central resistance and elastance; this was abrogated by VPA. Taken together, our data demonstrate that VPA is effective in reducing ozone-induced lung injury, inflammation and oxidative stress, and mechanical dysfunction. These findings may be useful in the development of therapeutics to treat oxidant induced lung injury. *Supported by Rutgers SURF/ASPET, NIH ES004738, AR055073, and ES005022.*

1110 Characterization of Acute Ozone-Induced Leukopenia through Flow Cytometric Quantification of Immune Cells

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Acute ozone (O_3) inhalation concurrently increases circulating stress hormones, corticosterone and epinephrine, and decreases leukocytes (leukopenia) in rats. These two biological events correlate with the activation of the neuroendocrine stress response. We hypothesized that glucocorticoid-induced apoptosis in circulating lymphocytes was responsible for O_3-induced leukopenia. To examine the dynamic changes in circulating leukocyte subpopulations we quantified live, apoptotic (early and late) and necrotic granulocytes, monocytes (classical and non-classical), and lymphocytes (B and T [T helper and T cytotoxic]) using flow cytometry (n=8) in 12-13 weeks old male WKY rats exposed to air or O_3 (0.0, 0.4 and 0.8 ppm) for (30 min, 1h, 2h and 4h). To evaluate if circulating changes correlated with those taking place in immunological organs where maturation of leukocytes occurs, thymus and spleen cell populations were also analyzed. Generally, for those leukocyte subpopulations affected by O_3 exposure, the responses were dose and time dependent. Circulating lymphocyte count was significantly decreased after O_3 exposure (0.8 ppm, 2h and 4h). At 4h, this decrement was more extensive in B than in T cells (~50% and ~20% [for both T helper and T cytotoxic]). Circulating classical monocytes, also known as inflammatory monocytes, decreased after 4h of exposure (~60% and ~80% at 0.4 and 0.8 ppm respectively). Circulating granulocytes increased after 30 min of O_3 exposure (at both 0.4 and 0.8 ppm) but tended to decrease after 2h of exposure in 0.8 ppm O_3-exposed rats suggesting a rapid mobilization of these cells into peripheral organs. No significant ozone-induced changes were detected in cell populations for thymus and spleen at any time point. Also, the assessment of cells undergoing apoptosis or necrosis yielded no significant alterations in blood, thymus or spleen. Our results indicate that O_3-induced leukopenia is not mediated by cell death of lymphocytes or any other subpopulation, but rather by the mobilization of such cells. This study provides insights on how O_3 exposure favored the decrease of specific leukocytes subpopulations such as classical monocytes and B lymphocytes that could explain the nature of O_3-induced immune effects in the respiratory system. *This abstract does not reflect the US EPA policy.*

1111 Lung Epithelial Cell Susceptibility Driven by Surfactant Protein-C Mutation Enhances Ozone-Induced Toxicity

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Ozone is a ubiquitous air pollutant that causes moderate parenchymal stress and monocyte/macrophage inflammation in healthy individuals. These effects are heightened in susceptible populations including the elderly and patients affected by interstitial lung disease (i.e., pulmonary fibrosis, PF). Pulmonary fibrosis is a degenerating disease characterized by progressive disruption of the alveolar architecture interspersed with episodes of acute inflammatory exacerbations. Mutations in the alveolar epithelial type-2 cell-specific Surfactant Protein C (SP-C) gene (*SFTPC*) have been identified in a subset of PF patients, with the Ile->Thr substitution at position 73 (SP-C^{I73T}) as the most prevalent. To investigate the susceptibility of SP-C mutant populations to acute ozone exposure, we leveraged a novel inducible SP-C^{I73T} transgenic mouse. Low-level SP-C^{I73T} expression produces moderate enlargement of the alveolar septae, AT2 cell hyperplasia, and minor inflammation beginning at 16wk and deteriorating with time. Conversely, SP-C^{I73T} induction results in extensive polycellular inflammation, decline in respiratory function and lung remodeling, distinctive features of acute exacerbations. Population RNA-sequencing and targeted cytokine analysis of bronchoalveolar lavage fluid show that AT2 cells initiate monocyte/macrophage recruitment and activation via canonical (CCL-2, CX_3CR1) and non-canonical (IL-5, Eotaxin, and CCL-17) pathways during SP-C^{I73T} acute exacerbations. Consistent with these findings, RNA-sequencing of $SiglecF^{lo}CD11b^{+}CD64^{-}Ly6C^{+}$ monocytes indicate highly inflammatory (*inos*, *Il-6*) and pro-fibrotic (*col1a1*, *col1a2*) phenotype. Pharmacologic (intravascular clodronate liposomes) and genetic ($CCR2^{ko}$ mice) monocyte ablation resulted in reduced inflammatory burden and improved survival following SP-C^{I73T} exacerbations. Acute low-dose ozone exposure (0.8ppm, 3h) of SP-C^{I73T} mice resulted in heightened alveolar septal disruption, edema and perivascular immune cell infiltrate compared to SP-C^{WT} cohorts. These responses were observed in mice undergoing acute exacerbations, as well as aged SP-C^{I73T} cohorts (52 wk) expressing low levels of mutant protein. Taken together, our findings highlight intimate crosstalk between epithelial and inflammatory monocytes during acute exacerbations. In addition, these data support the notion that epithelial dysfunction aggravates respiratory symptoms induced by ozone exposure. *Grant support: P30ES013508 (MFB), VA 1I01BX001176 (MFB), ES004738 (DL), ES005022 (DL).*

1112 Effects of Chronic Pulmonary Inflammation on Ozone-Induced Alterations in the Lung Microbiome

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Ozone (O_3) is a ubiquitous urban air pollutant known to cause pulmonary inflammation and constriction of the airways. The effects of O_3 are particularly severe in those with pre-existing lung disease, potentially via a "2-hit" mechanism involving alterations in the pulmonary microbiome. In these studies, we examined the effects of O_3 exposure on the microbiome in mice lacking *Sftpd* which exhibit chronic pulmonary inflammation. C57Bl6/J (Wt) mice and *Sftpd -/-* mice were exposed to air or O_3 (0.8 ppm, 3 hr); bronchoalveolar lavage fluid (BAL) was collected 24 hr later. Flow cytometry was used to identify lung macrophages (CD45+/SiglecF+/F4/80+) as resident alveolar macrophages (AM) (Cd11b-/Cd11c+) or inflammatory macrophages (IM) (Cd11b+/Cd11c+), or neutrophils (PMNs) (CD45+/SiglecF-/Cd11b+/Ly6G+) in BAL. Significantly fewer AM were detected in *Sftpd -/-* mice (75 ± 2.6%*) relative to Wt (91 ± 1.8%). O_3 exposure caused a greater loss of AM in *Sftpd -/-* mice (62 ± 2.9%*#) than Wt (88 ± 3.2; this correlated with a greater percentage of IM (9.8± 0.9% vs 2.2 ± 1.0%*). However, O_3 had no effect on these responses. Significant neutrophilia was observed in *Sftpd -/-* mice (8.9 ± 0.6%*#) compared to Wt (4.3 ± 0.6%#) after O_3 exposure. This was associated with increased disruption of the lung lining fluid. Thus, the BAL phospholipid content in *Sftpd -/-* was increased relative to Wt (51 ± 11 μg vs 8.23 ± 18 μg*). Moreover, this was exaggerated following O_3 exposure (89 ± 12 μg*) relative to Wt (31 ± 10 μg). As inflammatory cell activation is known to affect the pulmonary microbiome by regulating turnover, changes to the lung lining fluid alter the growth capacities of different microbes, we next profiled microbial diversity within the lung of these mouse strains and the impact of O_3 exposure. Baseline differences in microbial populations were observed between Wt and Sftpd -/- mice (3.8 vs 2.7 Simpson index). A loss in microbial diversity was observed in Wt mice (2.2) 48 hr following O_3 exposure, with an expansion in microbial species in *Sftpd -/-* mice (7.5). As the increase in diversity is proposed to occur as a result of the change in growth conditions, we investigated growing bacteria using stable isotope probing. A significant shift in the expanding microbial populations was detected in both strains following O_3 exposure. These data demonstrate that O_3 alters inflammatory cell activation and BAL lipid content and the pulmonary microbiome in a manner that is exaggerated by chronic inflammation. (*p<0.05 vs Wt, #p<0.05 vs air). *Supported by NIH Grants: HL086621, ES004738, ESO05022 and ES029254.*

1113 Role of PPARγ in the Resolution of Ozone-Induced Lung Injury

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Ozone is a ubiquitous urban air pollutant that causes airway inflammation and hyperresponsiveness in both healthy and susceptible populations. Macrophages play a role in ozone-induced lung injury by regulating the acute initiation and later resolution phases of the inflammatory response. The distinct activities of macrophages are mediated by subpopulations broadly classified as M1/pro-inflammatory and M2/anti-inflammatory that sequentially accumulate in injured tissues. RNA-seq analysis of alveolar macrophages revealed that exposure of mice to ozone resulted in alterations in inflammatory and lipid metabolism pathways, and down-regulation of PPARγ, a nuclear transcription factor important in M2 macrophage activation, inflammation resolution, and tissue repair. We hypothesized that administration of a PPARγ agonist would reduce ozone-induced lung injury by regulating macrophage activity and tissue repair. Female C57BL/6J mice were treated with rosiglitazone or vehicle control by daily intraperitoneal injection beginning 24 hr

prior to exposure to ozone (0.8 ppm, 3 hr) or air. Mice were euthanized 24, 48, and 72 hr post ozone. Bronchoalveolar lavage fluid (BAL) was collected and analyzed for total protein and phospholipid content. BAL was enriched for alveolar macrophages by gentle lung massage and isolated cells analyzed by flow cytometry and qPCR. Ozone caused increases in total protein and phospholipid content in BAL, consistent with lung injury. Rosiglitazone treatment resulted in decreases in BAL protein levels at 48 hr and phospholipid content at 72 hr when compared to ozone-only exposed mice, suggesting accelerated injury resolution. Flow cytometric analysis showed increases in both M1 and M2 macrophages in lungs of ozone-exposed animals throughout the time-course; reduced numbers of these cells were observed in rosiglitazone-treated animals at 72 hr. These results were consistent with reduced mRNA expression of pro- and anti-inflammatory genes Ptgs2 and Arg1, respectively, in macrophages at 72 hr. mRNA expression of Caveolin 1, a downstream target of PPARγ involved in lipid catabolism, was reduced in macrophages in response to ozone but sustained in rosiglitazone-treated animals. Collectively, these results suggest that PPARγ accelerates the resolution of ozone-induced lung injury by regulating inflammatory signaling and lipid metabolism in alveolar macrophages. *Supported by NIH Grants ES004738, ES005022, ES029254, ES007148, HL086621, and ES030984.*

PS 1114 The Influence of Acute Ozone Exposure and Hepatic Vagotomy on Glucose and Fatty Acid Metabolism

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Air pollution exposure has been linked to augmented incidences of metabolic diseases like obesity and insulin resistance. We have previously shown that acute ozone (O_3) exposure is associated with increased circulating stress hormones and impaired glucose and fatty acid metabolism. In a previous study, Affymetrix array data indicated 2335 genes changed after ozone exposure in healthy rats. Comparing these gene expression changes to a database of ~10K microarray comparisons from chemical exposure in rat liver demonstrated that ozone induced a set of glucocorticoid receptor-regulated genes, indicating the contribution of increased hypothalamic-pituitary-adrenal axis activation after ozone exposure. The aim of this study was to 1) characterize ozone-induced metabolic changes in the liver and 2) determine if these effects are altered by hepatic vagotomy that would reduce parasympathetic influence on metabolic processes. Male 12-week old Wistar-Kyoto (WKY) rats underwent sham surgery or hepatic vagotomy 4-6 days prior to air or ozone exposure (0.0, or 1.0 ppm; 6 hrs/day for 1 or 2 days). Glucose tolerance testing was performed immediately following the first day of ozone exposure, and circulating metabolic hormones and lipids were measured in the serum at both time points. Ozone-induced hyperglycemia, glucose intolerance, and increased circulating cholesterol, triglycerides, and leptin were sustained in vagotomized rats. Hepatic vagotomy did decrease circulating insulin in ozone exposed rats only. Liver global gene expression changes were determined using Illumina RNA-sequencing in sham and vagotomized rats. Inhibition of cholesterol biosynthetic genes after ozone exposure in healthy rats suggested suppression of SREBP2 consistent with increases in circulating cholesterol in ozone-exposed rats. In conclusion, ozone induced metabolic alterations were correlated with hepatic glucocorticoid activation, and vagus denervation exerted a modest influence on ozone-induced metabolic effects. *This abstract does not necessarily reflect US EPA policy.*

PS 1115 Effects of Ozone-Generated Microvesicles and MV-MiR-199a-3p on Inflammatory Lung Responses in Alveolar Macrophages

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Ozone is an urban air pollutant and highly reactive oxidant which generates free radicals and oxidizes cellular components. In addition to direct ozone-induced alveolar damage, evidence suggests that lung macrophages have displayed either a protective or destructive role in the lungs following ozone exposure. Extracellular vesicles (EVs) play an essential role in intercellular communication via the transfer of EV cargo. Accumulating evidence suggests that EVs regulate a variety of human diseases through their effects on cell-cell crosstalk. The purpose of this study was to investigate the role of ozone-induced EVs in macrophage activation and lung inflammation. Microvesicles (MVs), a sub class of EVs, are the main type of EV detected in broncho-alveolar lavage fluid. Using nanoFACS, we found that both air- and ozone-generated MVs are primarily derived from epithelial cells. Through functional assays, we found that treatment of mice *in vivo* with ozone-induced MVs upregulates the expression of multiple inflammatory cytokines. To delineate the mechanisms by which ozone-induced MVs induce macrophage classical activation or lung inflammation, we focused on the regulatory mechanism of miR-199a-3p, a microRNA which was significantly increased in expression within MVs after ozone exposure. To introduce miR-199a-3p mimics into MVs, a modified method of calcium chloride transfection was used. We found that MV-miR-199a-3p induces expression of IL-1β in macrophages. Previously, we have reported that epithelium-derived MVs can be taken by lung macrophages and transfer MV-miRNAs into the recipient cells. Interestingly, miR-199a-3p level in ozone-stimulated macrophages was highly upregulated compared to macrophages obtained from air-control mice, however, the level of precursor miR-199a-3p was not significantly increased. This result suggests that elevated mature miR-199a-3p level in ozone-induced macrophages results from MV-mediated delivery of mature miR-199a-3p. Collectively, ozone-induced MVs potentially lead to acute upregulation of inflammatory cytokines in both mice lung tissue and macrophages. We also uncovered that mature miR-199a-3p may be selectively-packaged into MVs after ozone exposure and transferred to recipient macrophages, ultimately resulting in pro-inflammatory activation of macrophages and lung inflammation.

PS 1116 Ozone Exposure Exaggerates Lung Inflammation in Adult Mice with Muco-Obstructive Airway Disease

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Airway surface liquid (ASL) layer is essential for the proper functioning of muco-ciliary clearance system. In addition, ASL layer acts as a physical barrier between reactive ozone and resident cells in airspaces including macrophages and epithelial cells. The lipids and proteins constituents of ASL are oxidized by ozone into reactive inflammatory mediators. We speculated that the inflammatory response to ozone is proportional to the concentration of lipids and proteins in the ASL layer. Accordingly, we hypothesized that the hyperconcentration of ASL constituents in muco-obstructive airways of mice will enhance their susceptibility to ozone-induced lung injury. Further, we hypothesized that *Scnn1b*-Tg exhibit exaggerated inflammatory responses to ozone inhalation. Here, the *Scnn1b*-Tg mouse model that manifest ASL dehydration and hyperconcentration was employed. Wild-type (WT) and *Scnn1b*-Tg adult mice were exposed to filtered air (FA) or 0.8 ppm of ozone for three weeks (4 hours/night). Total and differential cell count of bronchoalveolar lavage fluid (BALF) estimation, BAL cytokines estimation, histopathological analysis and macrophage activation were performed. Inflammatory cells as well as cytokines in BALF from FA-exposed *Scnn1b*-Tg mice were significantly higher as compared to both WT groups. Further, ozone exposed WT mice had significantly increased number of inflammatory cells and cytokines in BALF, and had exaggerated morphological activation of macrophages. These responses were heightened in ozone-exposed *Scnn1b*-Tg mice. Our ongoing analyses reveals that the latter group has increased mRNA message related to inflammatory responses. These data indicate that superimposition of ozone onto the ASL dehydration results in exaggerated inflammatory responses in the airspaces.

PS 1117 Pulmonary Impacts and Responsiveness to Ozone Inhalation after Exposure to an Environmental Allergen Mixture

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Multiple lines of evidence link exposure to air pollution with increased asthma-related morbidity and mortality. While several mechanisms have been postulated from experimental findings, many of the rodent asthma models fail to elicit features of chronic disease, bringing in to question pathophysiologic relevance. Recently, an approach using intra-airway exposure to a mixture of relevant environmental allergens, including house dust mite, *Aspergillus fumigatus*, and ragweed, has been shown to reliably elicit chronic features of asthma. The goal of this study was to determine the pulmonary and ventilatory impacts of short-term and chronic treatment with such an allergen mixture in female rats and assess respiratory sensitivity to a prototypical air pollutant. Female Wistar rats were repeatedly intranasally instilled with either the allergen mixture or saline alone. Pulmonary and systemic inflammatory markers were assessed in both short-term (2 weeks; ST) and long-

term (6 weeks; LT) allergen treatment cohorts. Shortly after the final allergen challenge, the LT cohort was exposed once for 2 hours to 0.5 ppm ozone. The allergen mixture caused an increase in bronchioalveolar lavage fluid (BALF) eosinophils and lymphocytes, interleukin (IL)-1 β, IL-4 IL-5, IL-10, tumor necrosis factor-alpha (TNF-α), and keratinocyte chemoattractant (KC)/growth-regulated oncogene (KC-GRO), as well as a decrease in interferon (IFN)-γ compared to saline in the ST cohort. No such changes were evident in the LT cohort. By contrast, ozone exposure in allergen-instilled rats, but not saline-instilled rats, in the LT cohort, caused an increase in BALF IL-13, IFN-γ and KC-GRO. Ventilatory data collected using double chamber plethysmography and histopathologic changes are currently being analyzed. In summary, mixed allergen exposure caused a lung inflammatory and cytokine response that shifts over time with repeated challenge, but that still confers exaggerated sensitivity to ozone after long-term allergen treatment. *This abstract does not reflect US EPA policy.*

1118 Acute Effect of Wildland Fire Smoke Exposure on Systemic Oxidative Stress among Wildland Firefighters in Midwestern United States

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Wildland firefighters (WFFs) repeatedly experience exposure to elevated levels of wildland fire smoke (WF smoke), which is composed of a wide variety of health-damaging compounds such as $PM_{2.5}$ (particulate matter with diameter less than 2.5 microns) and carbon monoxide. Previous studies reported that occupational WF smoke exposure is associated with increased systemic oxidative stress among the firefighters. However, such information is geographically limited to southeastern and western regions of the United States, and their personal occupational exposure level to $PM_{2.5}$ in WF smoke is much lower than the levels observed in the present study. The objective of the study is to investigate changes in oxidative stress (i.e. 8-isoprostane and 8-OHdG) in urine samples collected from WFFs after WF smoke exposure. Between 2016 and 2018, a total of 19 wildland firefighters (17 males and 2 females with aged 34.63 ± 7.21 years) employed by USDA-Wayne National Forest were recruited. Spot urine samples (n = 120) were collected from the subjects right before (pre-shift), immediately after (post-shift), and next morning of prescribed burn (burn days) and regular work shifts (non-burn days). Samples were collected on a total of 27 and 15 person-days on burn and non-burn days, respectively. Urinary 8-isoprostane was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Difference in cross-shift changes (i.e. pre vs. post, pre vs. next morning, and post vs. next morning) in systemic oxidative stress compared between burn and non-burn days was assessed using linear mixed effect model. About 70% of WFFs had an increased urinary 8-isoprostane level after WF smoke exposure during the prescribed burns. The levels of urinary 8-isoprostane at post-shift and next morning are 1.26 and 1.22-fold higher than pre-shift on burn days, respectively, although the increases are not significant ($p = 0.15$ and 0.50, respectively). Ratio of 8-isoprostane level at post to pre-shift on burn days is marginally higher than the ratio on non-burn days ($p = 0.07$). Results suggest that WF smoke exposure during the prescribed burns is capable of inducing acute oxidative stress response among the WFFs and such acute response could be sustained for at least 24 hours, presumably due to their elevated exposure to WF smoke.

1119 Particle Filtration Ameliorates Respiratory Suppression Induced by Smoldering Smoke Emissions from Burn Pit Materials

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Exposure to smoke from combustion of synthetic materials in municipal solid waste or military burn pits may be associated with reduced respiratory function or pulmonary inflammation, similar to effects observed after wildfire smoke exposures. We examined the comparative respiratory and inflammatory effects in mice of acute exposures to smoke generated by smoldering combustion of military burn pit-related materials, including plywood (PW), cardboard (CB), plastic (PL; a mixture of LDPE, HDPE, PET and PS), or a mixture (MX) of PW, CB and PL, and assessed the role of smoke particulate matter (PM) using HEPA filtration. Female Balb/cJ mice were exposed 1 hour on each of 2 consecutive days to whole or filtered smoke or clean air in a nose-only exposure tower. Combustion emissions contained ~40 (whole) or ≤ 0.2 (filtered) mg/m^3 PM for all four exposure types, while CO (range 35-75 ppm), other gases and volatile organic compounds were unaffected by filtration. Respiratory function was assessed by head-out plethysmography 20 min before, during, and 10 min after exposure. All unfiltered smoke had significant effects on respiration relative to pre-exposure, including expiratory time (Te; increased 164-265%), minute volume (decreased 45-60%), and breathing frequency (decreased 45-63%). Whole PL exposure had greater effects than CB, PW, or MX on at least 1 exposure day. HEPA filtration significantly improved breathing parameters during PW, PL, and MX exposures, but there were no significant differences between whole and filtered CB smoke exposures. The average increase in Te on both days for whole PW relative to pre-exposure (199%) was reduced to 82% with filtration, while Te increased 168% with whole CB and 148% during filtered CB. A few relatively small changes in lung lavage and complete blood count indices were detected 4 hours after final exposure to PL and MX, however real-time respiratory function was the more sensitive and robust indicator of these smoke exposure effects. Our data suggest that material type influences respiratory responses to burn pit combustion emissions and that PM filtration provides substantial but incomplete protection. *This abstract does not represent US EPA policy; DoD award #W811XWH-18-1-0731 to I.J.*

1120 Effects of Proteins and Humic-Like Substances on Iron-Mediated OH Radical Formation in Human Lung Fluids

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Inhalation of particulate matter is hypothesized to contribute to health effects by overproducing reactive oxygen species (ROS) and inducing oxidative stress. Fe(II) has been shown to contribute to ROS generation in simulated lung fluids containing physiological amounts of ascorbate. Atmospheric humic-like substances (HULIS) have been shown to chelate Fe(II) and significantly enhance ROS generation, but there have been no studies investigating this chemistry using physiological lung fluids derived from humans. Here, we investigate iron-mediated OH generation both from the dominant proteins that interact with iron in lung fluid, albumin and transferrin, and fulvic acid, a surrogate for HULIS. We find that albumin is capable of enhancing OH generation from inorganic Fe(II) and that transferrin attenuates this enhancement. We estimate the rate constants for albumin-Fe(II) and fulvic acid-Fe(II) mediated O_2 to O_2^- reduction (1.8 ± 0.1) M^{-1} s^{-1} (pH = 5.5, T = 37 °C), enhancements of about 20 and 30 times the rate for free iron. OH generation measured from fulvic acid-Fe(II) in human bronchioalveolar lavage fluid (n = 8) correlates reasonably well ($r^2 = 0.5$) with expected generation based on concentrations of Fe(II), albumin, transferrin, SRFA and ascorbate, suggesting that fulvic acid enhances OH formation, and that albumin and transferrin moderate the effect. We propose that fulvic acid, and thereby HULIS, is capable of mobilizing Fe(II) away from albumin thereby increasing the formation rate of O_2^- and ultimately of OH. *Supported by a Switzer Environmental Graduate Fellowship.*

1121 Contributions of TRPA1 and V3 to Wood Smoke $PM_{2.5}$-Induced Mucus Overproduction by Human Bronchial Epithelial Cells

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Transient receptor potential (TRP) ion channels are activated by a range of stimuli including changes in temperature, noxious chemicals, and flavors such as mint and cinnamon. There are 28 human TRPs, and the roles of different TRP channels in regulating the adverse effects of inhaled toxicants in lung epithelial cells are poorly understood. We have previously shown that chemicals present in wood smoke particulate matter < 2.5 µm ($WSPM_{2.5}$) collected from burning pine wood can selectively activate TRP channels TRPA1 and TRPV3. Using pine $WSPM_{2.5}$ as a model air pollutant, it was found that activation of both TRPA1 and TRPV3 contributes to $WSPM_{2.5}$ induced mucus overproduction, specifically the gel-forming glycoprotein MUC5AC, by primary human bronchial epithelial cells (HBECs). In addition, the stable overexpression of TRPV3 in HBECs amplifies $WSPM_{2.5}$ induced expression of MUC5AC, while knockdown of TRPV3 prevents the induction of MUC5AC. Similarly, activation of TRPA1 stimulates MUC5AC production while its inhibition attenuates MUC5AC overproduction. Accordingly, pine $WSPM_{2.5}$ treatment of C57BL/6 mice induced *Muc5ac* expression, which was not observed in *Trpv3*-/- mice. These results show that concurrent activation of TRPA1 and TRPV3 by WSPM plays a crucial role in promoting mucus overproduction and hypersecretion by HBECs. Identifying molecular targets and mechanisms responsible for mucus overproduction may facilitate the development of novel mucolytic treatments for acute inflammatory and chronic obstructive pulmonary diseases. *Support: ES017431 and ES027015.*

1122 High-Fructose Diet Alters Cardiovascular Function One Day after a Single Wood Smoke Exposure in Adult Wistar-Kyoto Rats

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Wildfires are extreme events that have a significant potential to harm both healthy individuals and those with underlying conditions like cardiovascular disease. We previously demonstrated that exposure to a single episode of wildfire smoke can alter homeostatic function and worsen cardiovascular responses in healthy animals. However, it is not well established how diet affects the cardiovascular response to wildfire smoke. High-fructose (HF) intake has been associated with the development of obesity, inflammation, increased oxidative stress, and autonomic imbalance. Therefore, this study examines the effects of a HF (30% fructose, 0% sucrose) diet on cardiovascular responses to wood smoke (WS) in Wistar-Kyoto rats. We hypothesized that a HF diet would alter basic metabolism, body composition and hemodynamic function of the heart, and worsen the subsequent response to WS. Eight-week old Wistar Kyoto rats were placed on either a HF or normal diet (ND) for seven weeks. Body composition was measured every three weeks for fat, lean, and fluid compartment percentages, and animal resting energy metabolism was analyzed using an indirect calorimeter. Each group was exposed to 0.5 mg/m^3 of flaming WS or filtered air (FA) for one hour. Twenty-four hours after the exposure, rats were anesthetized, and cardiac function was determined with echocardiography. There were no significant changes in body composition or metabolic rate in HF rats compared to the control. HF rats exposed to WS and FA had significantly lower cardiac output than the ND rats. However, there was no difference in ejection fraction between the groups, and fractional shortening was only marginally higher in the HF group exposed to FA. The ND group exposed to WS had a significantly higher stroke volume when compared to the control. Exposure to WS caused SDNN to increase and RMSSD to decrease in ND rats, on the other hand, HF rats exposed to WS only had an increase in the latter and showed a trend towards increased SDNN. There were no significant differences in frequency domain parameters. This data indicates that a HF diet alters baseline cardiovascular function even in the absence of metabolic or body composition changes in Wistar Kyoto rats and suggests that diet can predispose the cardiovascular system to adverse effects during and after a single exposure inhalation. *This abstract does not reflect US EPA policy.*

1123 Emission Toxicity of Spruce and Lignite Combustion in Residential Heating Using Thermophoretic ALI Exposure System

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Biomass combustion is one of the actions that can be taken to lower CO_2 emissions from residential heating. Even though European Union has been shifting more towards biomass, in some countries, solid fossil fuels are still used widely and even low-grade ones such as lignite are used in residential heating. Combustion emissions have many known health consequences, but it is not clear how coal compares to wood fuel in small scale combustion. In addition, the effect of atmospheric aging on the toxicity of emissions is not fully known. We studied spruce and lignite combustion emissions with and without aging on co-cultured cells in thermophoretic air-liquid interface (ALI) exposure system. Co-cultures consisting of alveolar epithelial A549 cells that were cultivated in basolateral side of inserts for 72 hours, and monocyte THP-1 cells, which are differentiated into macrophages prior transfer, are transferred into apical side 24h before exposure. Exposure were done on cell with medium containing HEPES buffer for an hour at air flow of 150ml/min. The medium was collected after exposure and fresh medium added. After 24h post-exposure both the changed medium and cells were collected for toxicity experiments. Toxicological analyses from cells were viability, mitochondrial superoxide, genotoxicity. Cell media were used for cytokine measurements. We found the cell viability being most affected by the aging process, whereas fresh samples showed no effect of the exposure. Mitochondrial superoxide analysis showed an increase after exposure to aged lignite smoke, compared to clean air and fresh lignite samples. CXCL1 analyzed from medium collected immediately after exposure showed aged emissions to activate more secretion, which was still detected after 24h post incubation period. Interestingly, there were no evident difference in genotoxicity between the fuel types or the aging of the emission. Together our results showed that lignite combustion samples present slightly higher responses in our exposures than wood combustion samples and the aging process increases the overall toxicity of the both combustion emission.

1124 Transient Receptor Potential Vanilloid-3 (TRPV3) and Ankyrin-1 (TRPA1) Regulate Wood Smoke $PM_{2.5}$-Induced Endoplasmic Reticulum Stress and Cytotoxicity in Lung Epithelial Cells

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Inhalation of particulate pollutants can promote and exacerbate pulmonary inflammatory diseases. A frequently cited effect of particles on lung epithelial cells is disruption of endoplasmic reticulum (ER) homeostasis, causing a pathological stress response (ERS). Transient receptor potential (TRP) ion channels often localize to the ER of lung epithelial cells where they regulate ER calcium homeostasis, and thus ERS and associated effects on cell functions and viability. Wood smoke $PM_{2.5}$ (WSPM) activates TRPV3 and TRPA1, and this study shows that TRPV3 is located on the ER of primary human lobar bronchial epithelial cells (HBECs). Further, treating cells with pine WSPM resulted in a time-dependent activation of all regulatory arms of the ERS response; PERK/EIF2αK3, ATF6α, and IRE1. Interestingly, HBECs treated with pine WSPM upregulated TRPV3 mRNA transcripts and downregulated TRPA1 transcripts, and inhibition of TRPA1 partially protected cells from WSPM-induced ERS and cytotoxicity, while inhibition of TRPV3 exacerbated ERS response and cytotoxicity. These data suggest that activation of TRPA1 plays a direct role in initiating ERS and TRPV3 negatively regulates ERS. Further, overexpression of TRPV3 conferred resistance to ERS and associated downstream cellular events, including cell cycle arrest at G2. The protective effect on ERS was most evident with WSPM, which also activates TRPV3. Collectively, these results suggest that TRPV3 and TRPA1 play opposing roles in mediating the WSPM-induced ERS response. These findings provide new insight on the physiological functions of TRPV3 and TRPA1 in the lung epithelium, and expand our understanding of mechanisms by which HBECs regulate life and death signals when exposed to cytotoxic agents such as WSPM.

1125 Lung Toxicity of Particulate Matter from Smoldering Combustion of Simulated Military Waste

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A burn pit was a common way to get rid of waste at the United States military bases in Iraq and Afghanistan between 2001 and 2011. Consequently, many deployed military and civilian personnel were exposed to burn pit smoke. While there is a higher prevalence of respiratory conditions in veterans returning from service in these locations, the relationship to burn pit smoke exposure is not well understood. We used a quartz-tube furnace coupled to a multistage cryotrap system to collect smoke particulate matter (PM) from a simulated smoldering military burn pit. We burned four materials common to burn pits: plywood (military spec wooden box), cardboard (military spec weather resistant box), plastic (a mixture of LDPE, HDPE, PET and PS), and mixture (a mixture of plywood, cardboard and plastic). PM in smoke was chemically analyzed and assessed for lung toxicity in mice via oropharyngeal aspiration and mutagenicity in *Salmonella* strain TA98 +/-S9. Combustion efficiency ranged from 68% to 78%. The plastic burn simulations emitted the highest PM mass, followed by the mixture, the plywood and the cardboard. PM was comprised of 42% to 64% total carbon and very low levels of polycyclic aromatic hydrocarbons (<0.1 wt%). On an equal mass basis (100 µg of PM condensate), PM from burning plywood induced small but significant lung toxicity (neutrophil influx) at 4-h post-exposure. A significant alteration in lung ventilatory parameters was also observed in mice exposed to the plywood and cardboard smoke PM at 4-h post-exposure. No effects on the lungs were observed with exposures to other smoke PM. None of the PM samples showed significant mutagenic responses. Previously we have reported that lung toxicity of biomass smoke PM is significantly greater for flaming than smoldering combustion, on an equal mass basis (Kim et al., EHP 126: 017011, 2018). Similarly, here we demonstrate that PM in smoke derived from smoldering combustion (low temperature and flameless) of different burn pit materials caused minimal or no lung toxicity and mutagenicity following acute exposures. Further studies are needed to evaluate lung toxicity of flaming

combustions of these and other materials by both instillation and inhalation routes of exposures. *This abstract does not represent US EPA policy; DoD Award # W81XWH-18-1-0731 to I.J.*

1126 Analysis of Long-Term Effects from Wildfire Smoke in a Rural Montana Community

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As wildfires continue to affect the Western United States, the need for expanding research on the effects of wildfire smoke exposure is warranted. Historically, studies on the effects of wildfire smoke exposure have been retrospective analyses of visits to providers, emergency departments, and hospitals, using ICD codes for cardiovascular and respiratory designations. The present study is designed to assess the long-term effects of wildfire smoke through a series of screenings considering physiological, psychological, and social health dynamics. This interprofessional research is focused primarily on the rural community of Seeley Lake, MT, which experienced unprecedented levels of smoke exposure from July 31 to September 18, 2017, with a daily average of >220 ug/m3. From 2017 to 2019, multiple health screenings were performed in Seeley Lake. In the first three years of this study, 103 different participants have been screened. Based on spirometry data, participants showed decreased lung function in 2018, with a slight improvement, but still significantly decreased, in 2019. The results also illustrate that the health effects are more pronounced in individuals above the age of 65, where their respiratory values were consistently below predicted values. Another component of the research consisted of two validated behavioral screenings, which calculated either mood states or depression scores for each individual. The mood state screenings completed on the day that the smoke cleared in 2017 showed more positive results, while subsequent years showed decreased scores. In contrast, the depression screening which addresses a larger time frame showed that people were more depressed in 2017 (while the fire was burning), and were less depressed in 2018 and 2019. This research highlights how inhalation toxicology can be applied to assess the prolonged effects of wildfire smoke exposure in rural communities.

1127 Burn Pit Components Exhibit Variable Toxicity on Human Bronchial Epithelial Cells

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Burn pits are areas designated for disposal of military waste by open air combustion without proper waste management protocols. This system was prevalent in Afghanistan and Iraq from 2002-2009, where an estimated 60,000 pounds of solid waste was burned daily. Exposure to toxic compounds from burn pit synthetic waste combustion may be associated with airway diseases and decreased respiratory function. We hypothesized that burn pit condensates would have a dose-dependent cytotoxic effect on human bronchial epithelial cells *in vitro*. Military waste materials, such as plywood, plastic, cardboard, and mixtures of these three components with and without diesel fuel were combusted under controlled conditions in quartz tube furnace system and emissions were collected in a serial cryotrap system. A solvent exchange was performed to transfer the emission condensates into cell culture media. Human donor bronchial epithelial cells were cultured on 96-well plates and co-incubated with the various condensates at 0.25, 0.5, 1,5, 10, 25, 50, 75, and 100 ug/cm^2 particulate concentrations for 24 hours. Cytotoxicity was assessed on a fluorescent plate reader using lactate dehydrogenase and adenylate cyclase assays. Plastics exhibited the greatest cytotoxic response, followed by the mixture and mixture containing diesel, with the least cytotoxicity seen in cells exposed to condensates from plywood and cardboard emissions. RNA analysis revealed exposure to condensates altered inflammatory (IL-8) and stress (HMOX-1) responses, with the mixture having the greatest effect. These results indicate that materials commonly disposed of in burn pits can be cytotoxic to airway epithelial cells and can possibly cause adverse respiratory health effects.

1128 Molecular Mechanisms of Wood Smoke Exposure in an Organotypic Model

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Wood smoke exposure causes airway inflammation and oxidative stress, which contributes to cardiopulmonary disease and early mortality; however, the molecular mechanisms responsible for these adverse effects are unclear. To date, epithelial cell monocultures have been widely used for identifying the molecular mechanisms involved in toxicity. While epithelial cells serve as the barrier between the host and the environment, they are only one of many cell types within the airway that cooperatively affect lung function. We hypothesized that trans-epithelial (TE) exposure to wood smoke condensate (WSC) would induce both the oxidative stress and pro-inflammatory response in airway fibroblasts. To test this hypothesis, we developed a Transwell-based *in vitro* organotypic model that recapitulates both *in vivo* cellular architecture and intercellular signaling. The model consists of an apical epithelial cell layer (16HBEs) and a basolateral fibroblast cell layer (IMR90s). Primary human bronchial epithelial cells and primary human lung fibroblasts are used when applicable. We assessed epithelial cell barrier integrity via trans-epithelial electrical resistance, fluorescein-labeled dextran permeability, and tight junction protein immunofluorescent staining. Importantly, the epithelial cell layer displays a high electrical resistance, low compound permeability, and polarized tight junction proteins. We then investigated the effects of TE WSC exposure on the kinetics of gene expression and protein expression for pro-inflammatory and oxidative-stress responsive genes. We found an increase in pro-inflammatory transcript expression (e.g. IL-8, IL-6), and an increase in oxidative-stress responsive genes (HMOX-1, NQO1). Changes at the protein level reflect transcript levels with peak protein expression (e.g. HMOX-1, 8-10 hours) following peak transcript expression (e.g. HMOX-1, 4-6 hours). Next, we examined the effects of repeat TE WSC exposure on airway fibroblasts. Preliminary data suggest differential gene expression between single exposure and repeat exposure. Differentially expressed genes display either a tolerance phenotype (e.g. HMOX-1) or a susceptibility phenotype (e.g. IL-8). Our results indicate an active role for airway fibroblasts in the toxicological response to wood smoke exposure, and facilitate new insights into the molecular mechanisms of toxicity.

1129 Diesel Exhaust Particle Exposure Causes Inflammatory Responses in Heart and Brain Despite Subtle Lung Inflammation

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Air pollution is a significant threat to public health and even short-term exposures pose an acute cardiopulmonary risk to susceptible individuals. Emissions from gasoline and diesel engines are major contributors to airborne fine and ultrafine particulate matter (PM) in the urban environment. These particles are readily deposited in the lung and can have a large surface area to which organics adsorb easily. However, the pathways by which an insult to the lung can lead to adverse cardiovascular outcomes are not well understood. This study was designed to test the hypothesis that lung inflammation induced by diesel exhaust particles (DEP) - used here to model submicrometer carbonaceous exhaust PM - initiates a systemic inflammation in heart and brain. Mice (C57 BL/6, male, 10-12 weeks) were exposed to filtered air or DEP (NIST SRM 1650b, 0.5 mg/m^3, 4 hr/day, 1-4 days). Bronchoalveolar lavage fluid (BALF) was collected 24 hr after the last exposure (1, 2, and 4 days) to measure the number and type of cells. Following exposure, DEPs were visible in alveolar macrophages of BALF. DEP inhalation significantly increased the number of macrophages, but not polymorphonuclear neutrophils. Nod-like receptor family pyrin domain-containing 3 (NLRP3), interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) gene expression were measured as indicators of the inflammatory response in lung, heart and brain tissue by real-time PCR. NLRP3 and IL-1β gene expression in heart, but not in lung, was elevated in response to DEP exposure. DEP inhalation also induced increases of IL-6 and MCP-1 gene expression in brain. These exciting results indicate that DEP can induce significant inflammation at distant sites despite little inflammation in lung tissue. Further studies are required to prove the specific role of alveolar macrophages and to identify the mechanisms for cardiovascular inflammation after DEP exposure. *Funding sources: 1R01HL134910-01 and P30ES001247.*

1130 Urban $PM_{2.5}$ Pollution Disrupts Cellular Physiology in Human Lung Cells: Role of the Unfolded Protein Response

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Ambient particulate matter with a mean diameter of ≤2.5μm ($PM_{2.5}$) poses a serious public health risk, in part, because $PM_{2.5}$ can penetrate the deep lung, enter general circulation, and be widely distributed throughout tissues. Epidemiological studies have consistently associated exposure to ambient $PM_{2.5}$ air pollution to increases in all-cause mortality, cardiopulmonary and cardiovascular disease, stroke, diabetes, cancer, and Alzheimer's disease. The normally picturesque Cache Valley of Northern Utah frequently experiences some of the highest $PM_{2.5}$ concentrations in the United States. However, the

exact mechanism(s) of Cache Valley's $PM_{2.5}$ (CVPM) toxicity have yet to be fully characterized. We recently demonstrated that CVPM exposure is associated with endoplasmic reticulum (ER) stress, which triggers the unfolded protein response (UPR), a highly conserved stress-response mechanism. The purpose of this study was to focus on the dynamics of CVPM-elicited ER stress and UPR in cultured human lung (BEAS-2B) cells exposed to CVPM (1µg/mL and 12µg/mL; 24 hours). All experiments were conducted in parallel with diesel exhaust particles (DEP) as a positive control. RNA sequencing with KEGG pathway analysis confirmed significant upregulation (FDR adjusted p=0.05) in genes strongly associated with UPR activation, such as *BiP/GRP78*, *PERK*, *IRE1*, and *ATF6*. Flow cytometry revealed that CVPM (12µg/mL) caused significant cellular effects related to UPR activation, including reductions in mitochondrial membrane potential and alterations in intracellular Ca^{2+} homeostasis, as evidenced by a significant influx of Ca^{2+} in the cytosol and mitochondria, likely from the ER network. In most experiments, 1µg/mL DEP elicited similar results to CVPM at 12µg/mL, suggesting that CVPM is less potent than DEP. Taken together, these results support our hypothesis that a principal toxic mechanism of CVPM pollution involves ER stress and the UPR. *This research supported by the Marriner S. Eccles Foundation, GE Healthcare, and Utah State University.*

1131 Diesel Exhaust Particles Reduce Airway Epithelial Barrier Integrity through a Reduction of the Tight Junction Protein Tricellulin

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Early life exposure to airborne particulate matter (PM) has been increasingly linked to the development of asthma. While changes in the expression of junctional proteins in lung epithelium has been seen in asthmatics, the impact of PM on their expression has received little attention. We investigated whether exposure to diesel exhaust particles (DEP), a major component of PM, would affect epithelial barrier function by reducing the expression of the tight junction protein Tricellulin. Standard reference material 2975 Diesel Particulate Matter (DEP) was purchased from NISTand suspended inculture media. Monolayers of the human bronchial epithelial cell line 16HBE14o- were grown on collagen coated Transwell inserts and treated with 25 µg/cm^2 DEP for 6 hours. Changes in barrier function were assessed by measuring transepithelial electrical resistance (TEER) and permeability of 4 kDa FITC-Dextran. Changes in tight junction protein levels were assessed by Western blotting of whole cell lysates. Neonatal Balb/c mice (postnatal day 4-7) were exposed to 255±50 µg/m^3aerosolized DEP or filtered air for 2 hours per day for 5 consecutive days, and sacrificed 2 weeks later. Lungs were collected, homogenized and analyzed by Western blot or RT-qPCR. Six-hour treatment of 16HBE14o- cells with DEP significantly reduced epithelial barrier function as measured by reduced TEER (584±54 versus 994±33 Ohms, n=5, p<0.05) and increased permeability to 4 kDa FITC-Dextran (26.0±1.8 versus 15.7±0.7 ug, n=5, p<0.05). These changes coincided with a significant reduction of Tricellulin protein as measured by Western blot (67±8% normalized band intensity versus vehicle, n=5, p<0.05). Neonatal Balb/c mice (pnd 4-7) exposed to aerosolized DEP presented with a significant reduction in Tricellulin in the lung two weeks post exposure as measured by Western blot (65±20% normalized band intensity versus filtered air, n=7, p<0.05) and RT-qPCR (63±26% of filtered air mRNA expression, n=12, p<0.05).Taken together, exposure to DEP caused a significant reduction in the expression of the tight junction protein Tricellulin. This reduction correlates with significant reduction in barrier function *in vitro* as measured by TEER and permeability to 4 kDa FITC-Dextran. Neonatal exposure to DEP caused a lasting reduction of Tricellulin at both the mRNA and protein level, suggesting early life exposure to DEP may cause a stable change in lung barrier structure and function.

1132 p.p1 Oxidative Potential of Particulate Matter and Generation of Reactive Oxygen Species in the Epithelial Lining Fluid p.p1

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p.p1 Reactive oxygen species (ROS) play a central role in adverse health effects of atmospheric particulate matter (PM). Respiratory deposition can lead to the formation of ROS in the epithelial lining fluid due to redox reactions of PM components with lung antioxidants. As direct quantification of ROS is challenging, PM oxidative potential is more commonly measured using antioxidant surrogates including dithiothreitol (DTT) and ascorbic acid, assuming that the decay of surrogates corresponds to ROS formation. However, this assumption has not yet been validated and the lack of ROS quantification in the respiratory tract causes major limitations in evaluating PM impacts on oxidative stress. By combining field measurements of size-segregated chemical composition, a human respiratory tract model, and kinetic modeling, we quantified production rates and concentrations of different types of ROS in different regions of the epithelial lining fluid by considering particle-size-dependent respiratory deposition. The extrathoracic region is found to have higher ROS concentrations compared to the bronchial and alveolar regions. While H_2O_2 and O_2^- production is governed by Fe and Cu ions, OH radicals are mainly generated by organic compounds and Fenton-like reactions of metal ions. In winter when affected by biomass burning, model comparisons suggest that humic-like substances (HULIS) contribute to ROS formation substantially. We found that PM oxidative potential is a good indicator of chemical production of H_2O_2 and O_2^-, but does not represent OH generation. These results provide rationale and limitations of the use of oxidative potential as an indicator for PM toxicity in epidemiological and toxicological studies.

1133 Particulate Matter ($PM_{2.5}$) Exposure Promotes Myofibroblast Differentiation through an NF-κB Dependent Mechanism

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Exposure to particulate matter has been linked to deleterious health outcomes including lung cancer and asthma. Epidemiologic studies show that exposure to $PM_{2.5}$ leads to a worsening of Idiopathic Pulmonary Fibrosis (IPF), a deadly lung disease of unknown etiology. Recent work suggests that exposure to $PM_{2.5}$ promotes the development of pulmonary fibrosis *in vivo*. However, the mechanism by which $PM_{2.5}$ contributes to the development of IPF is unknown. Here, we seek to elucidate the mechanism by which $PM_{2.5}$ promotes myofibroblast differentiation, an important process in the development of pulmonary fibrosis. A cell line of primary human lung fibroblasts were cultured and exposed to varying concentrations of $PM_{2.5}$ (.001 to 10 µg/cm^2) for up to 6 days. Cells were treated with $PM_{2.5}$ obtained either from the National Institute of Standards and Technology (collected in the Washington, DC area in 1976-1977; DC-$PM_{2.5}$), or from manual samplers that collected $PM_{2.5}$ from the rooftop of Peking University in Beijing, China (B-$PM_{2.5}$) in 2015. Cell lysates were analyzed for the expression of smooth muscle actin (SMA), fibronectin, and collagen. To determine the signaling mechanisms, each exposure was preceded by the addition of an NF-κB inhibitor, an AhR inhibitor, an antioxidant (N-acetylcysteine) or a vehicle control. A single exposure to either DC-$PM_{2.5}$ or B-$PM_{2.5}$ did not induce any measurable change in α-SMA, a marker of myofibroblast differentiation. However, when cells were exposed to repeated low doses of DC-$PM_{2.5}$, we observed an increase in α-SMA protein expression. The effect of B-$PM_{2.5}$ was similar, but occurred at higher doses. The $PM_{2.5}$ induced increase in α-SMA protein expression was blocked by pretreatment with an NF-κB inhibitor, but not by the AhR inhibitor or by N-acetylcysteine. Exposing fibroblasts directly to $PM_{2.5}$ *in vitro* resulted in myofibroblast differentiation. These changes occur in response to longer, repeated exposures but not in response to shorter exposure periods, or to a single $PM_{2.5}$ exposure, suggesting that longer exposure durations with repeated exposures may be necessary to accurately model chronic disease. The necessity of repeated exposures indicates that the initial $PM_{2.5}$ exposure may sensitize the fibroblasts to further environmental insults. The effectiveness of an NF-κB inhibitor in blocking these effects implies that $PM_{2.5}$ is acting through NF-κB signaling to promote myofibroblast differentiation. *This work was supported by HL127203 from NHLBI.*

1134 Ambient Particulate Matter-Induced Cardiac Mitochondrial Damage in C57/B6 Mouse

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Ambient particulate matter-induced cardiac mitochondrial damage in C57/B6 mouse Ambient particulate matter exposure is associated with elevated cardiovascular risk in general population, and mitochondria damage accounts for a large part of cardiotoxicity. To evaluate the potential cardiac mitochondrial damage following particulate matter (PM) exposure, an individual ventilated cage (IVC) system is utilized to expose C57/B6 mouse to either ambient air with particulate matter pollution (From 2018-2019 winter, Shijiazhuang, China), or filtered clean air for 21 or 42 days. To explore the potential molecular mechanism, WY 14,643 and GW6471, agonist and antagonist for the important fatty acid metabolism modulator, peroxisome proliferator-activated receptor alpha (PPARa), were administered to the animals along with the ambient PM exposure. At desired time points, heart tissues were collected, mitochondria were isolated, and then the mitochondrial membrane potential was assessed. Additionally, electron microscopy was utilized to visualize the mitochondria in myocardium. To explore the role of PPARa signaling, co-immunoprecipitation was performed to assess the association between PPARa

and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). The results indicated that exposure to ambient PM for 21 or 42 days effectively abolished the mitochondrial membrane potential. Decreased mitochondria quantity in myocardium and structural damage were also observed. PPARa agonist WY 14,643 co-treatment alleviated such changes, while PPARa antagonist GW6471 seemed to exacerbated the toxicities. Co-immunoprecipitation revealed decreased association between PPARa and PGC1a following exposure to ambient PM, while cotreatment with WY14,643 restored the association. In summary, ambient PM exposure induced mitochondria damage in C57/B6 mouse myocardium, including abolished membrane potential, decreased mitochondria quantity and structural damage. The underlying mechanism is associated with interruption with PPARa signaling pathway. *This work was supported by National Natural Science Foundation of China (Grant No.81872591, 91643203, 81502835).*

PS 1135 A Systematic Review and Analysis of Personal and Ambient $PM_{2.5}$ Measurements: Implications for Epidemiological Studies

S. R. Boomhower, J. E. Goodman, W. Li, and C. M. Long. *Gradient, Boston, MA.*

In epidemiology studies, ambient measurements of $PM_{2.5}$ (*e.g.*, from central-site outdoor air monitors) often are used as surrogates for personal exposures. However, estimating personal $PM_{2.5}$ from ambient measurements introduces uncertainty, and it is unclear the degree to which ambient $PM_{2.5}$ reflects personal exposures. We conducted a systematic review and statistical analysis of epidemiology studies to determine the extent to which ambient $PM_{2.5}$ is correlated with personal $PM_{2.5}$. We conducted a literature search in PubMed and Scopus for peer-reviewed studies reporting both personal and ambient measurements of $PM_{2.5}$ in North America published between January 1, 2009, and September 4, 2019. Two independent reviewers completed data extraction, which included recording geographic variables, sample characteristics, ecological variables, ambient $PM_{2.5}$ measurements, personal $PM_{2.5}$ measurements, and ambient-personal $PM_{2.5}$ correlations. Twenty-three studies were identified. Overall, personal $PM_{2.5}$ concentrations were higher than ambient concentrations. The median personal $PM_{2.5}$ concentration was 17.9 $\mu g/m^3$ (range: 2.0-92.2 $\mu g/m^3$), and the median ambient $PM_{2.5}$ concentration was 15.8 $\mu g/m^3$ (range: 6.0-33.3 $\mu g/m^3$). There was a moderate-to-strong relationship between personal and ambient $PM_{2.5}$ concentrations; median personal-ambient $PM_{2.5}$ correlation coefficients were 0.57 (range: 0.09-0.83). Stratified analyses suggest that geographic and other variables may influence the relation between personal and ambient $PM_{2.5}$. For example, studies that report controlling for environmental tobacco smoke (ETS) reported an approximate personal-ambient correlation of 0.78 on average, whereas studies that did not measure or did not report measuring ETS reported an approximate personal-ambient $PM_{2.5}$ correlation of 0.09 on average. Our study informs the interpretation of both past epidemiology studies in which health effects were associated with ambient $PM_{2.5}$ and future studies with regard to accounting for error in estimating $PM_{2.5}$ exposures.

PS 1136 Chemical Composition across $PM_{2.5}$ Filters: Potential Impacts for $PM_{2.5}$ Toxicology Research

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Toxicology research into the impacts of exposure to air pollution often requires the extraction of fine particulate matter ($PM_{2.5}$) from filters. In order to generate relevant toxicity data, there is a growing interest in validating that laboratory procedures create samples that are truly representative of samples collected from the environment. The ability to run multiple analyses on the same filter sample is beneficial to provide a robust characterization of $PM_{2.5}$ both from a compositional and toxicological viewpoint. Frequently, $PM_{2.5}$ filters are cut into pieces to allow for different methods of analysis, some of which destroy the sample like inductively coupled plasma mass spectrometry (ICP-MS). The goal of our study is to identify if portions of the same filter have similar elemental concentrations and thus provide information on if splitting a single filter for chemical analysis and toxicology testing provides data that can be used for associations between the two data sets. We will cut $PM_{2.5}$ collected filters (n=15) into 4 equal portions and analyze each portion for elements (n ~ 25) via ICP-MS. We anticipate that there will not be a significant difference in elemental concentrations between the quadrants from the sample filter. Our preliminary data from testing two sample filters split into quadrants for elements (n=14) mainly supports this. However, we observed significant differences between arsenic concentrations on different quadrants of the same filter. We are in the process of continuing to investigate these findings and the possible implications for toxicology testing. Our study will demonstrate the feasibility of allocation of the different portions of a $PM_{2.5}$ filter for multiple analyses and the potential impacts on $PM_{2.5}$ toxicity findings.

PS 1137 Toxicological Evaluation of Exhaust Emissions from Light-Duty Vehicles Using Different Fuel Alternatives in Sub-Freezing Conditions

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Sponsor: M. Viluksela

Emissions from road traffic are under constant discussion since the they are still posing a major threat to human health despite of the stricter emission targets and regulations. Although, the new passenger car regulations have been very effective towards the particulate matter (PM) emissions, the aged car fleet remain as a large source of the PM emissions. Moreover, PM emissions from multiple new type of bio-based fuels are still uncertain and the sub-zero running temperature has shown to affect the emissions. Overall, toxicological studies on these PM emissions and effect of temperature conditions are inadequate. In the present study we show that exhaust gas PM from four newly regulated passenger cars fueled by different fuels in sub-zero temperature, induce toxicological responses *in vitro*. We used exhaust gas volume-based PM doses to give us better insight on the real-life exposure and included one older diesel car to estimate the efficiency of the new emissions regulations. From the newly regulated cars, one fueled with gasoline had the both highest exhaust PM concentrations and toxicological responses, while the one fueled by higher ethanol blend resulted in slightly lower exhaust gas PM concentrations and notably lower toxicological responses compared with gasoline exhaust PM. Modern diesel and compressed natural gas fueled vehicles had overall the lowest exhaust PM concentrations and toxicological responses. Results of the present study show that toxicity of the exhaust gas PM differ by the fuels used. Additionally, the PM emissions and their toxicity were vastly higher from older diesel car, indicating the efficiency of the new emissions regulations.

PS 1138 A Comparison of Lung Particle Deposition and Clearance in $ApoE^{-/-}$ Mice versus Normal and Heterozygous Littermates

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Air pollution and particulate matter (PM) has been associated with increased incidences of cardiovascular disease leading to increased daily mortality and hospital admissions. Apolipoprotein E-deficient mice ($ApoE^{-/-}$) are a well-studied mouse model for cardiovascular disease and atherosclerosis due to the buildup of cholesterol particles in their blood. However, data now suggests that high non-HDL cholesterol may cause disordered trafficking of cholesterol and phospholipids throughout the lung thereby altering immune cell clearance rates bringing into question the efficacy of $ApoE^{-/-}$ mice in inhalation exposure studies. Therefore, the objective of this study is to quantitate particle clearance of inhaled 0.02µm and 1µm fluorescent microspheres in $ApoE^{-/-}$ mice compared to their wild type and heterozygous littermates while also determining the levels of immune cells residing pre- and post-exposure in each animal variant. Male mice (n=8/cohort) were exposed to re-aerosolized 0.02µm and 1µm fluorescent microspheres for 3 hours per day for a total of 4 days. The lungs, lymph nodes, and plasma of all animals were collected one hour following the last exposure period and prepared for subsequent cell sorting and immunohistochemistry (IHC) analysis. Our hypothesis was that $ApoE^{-/-}$ animals will have the same deposition but less clearance of the 0.02 µm and 1 µm fluorescent microspheres from the lung when compared to heterozygote and wild type littermates. Data suggests that there are differences between $ApoE^{-/-}$ mice when compared to their wild type littermates, and it has been demonstrated that alveolar macrophages eat 1µm fluorescent microspheres in *in vitro* experiments. The particle deposition vs. particle size was calculated using Multiple Path Particle Dosimetry (MPPD) for mouse and showed that 0.02 µm and 1µm fluorescent microspheres will deposit 44% and 2% of the microspheres in the pulmonary region, respectfully. Determining accurate clearance rates for the pulmonary cells this hyperlipidemic mouse model is extremely useful when ultimately interrogating whether an inhaled toxic substance can adequately trigger downstream immune responses.

1139 Chronic $PM_{2.5}$ Exposure-Mediated Recruitment of Bone Marrow-Derived Macrophages: Impact on Pulmonary Immune Homeostasis and Inflammation

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Chronic exposure to particulate matter air pollution <2.5µ ($PM_{2.5}$) has been linked to chronic cardiopulmonary disease. The impact of $PM_{2.5}$ on proliferation and maintenance of tissue-resident macrophages (MΦ) in the lung and the contribution of bone marrow-derived monocytes is poorly understood. Chimeric mice were developed by lethal irradiation (1,000 cGy) of chest shielded recipient (CD45.2) mice (to protect resident cells in lung) followed by Busulfan (i.p., 30mg/kg) and bone marrow (BM) transplantation from donor (CD45.1) mice. Following recovery (8 weeks), mice were exposed to concentrated ambient $PM_{2.5}$ (CAP) or filtered air (FA) 6-8 hours/day, 5 days/week using a *Versatile Aerosol Concentrator and Exposure System* for 4 and 32 weeks. Multi-color flow cytometry (10 color panel) was used to quantitate the myeloid cell subsets in the lungs and monocyte subsets in blood/spleen/BM and proliferation/apoptosis of alveolar MΦ (AMΦ, Siglec $F^{hi}CD11c^{hi}$). Flow sorted lung AMΦ (of CD45.2 & CD45.1 origin) and monocytes were used for RNA-seq analysis. Four weeks of $PM_{2.5}$ exposure resulted in higher CD45.1 $Ly6c^{hi}$ proinflammatory monocytes (2.5-fold, $p<0.05$) in the CD45.2 lungs of $PM_{2.5}$ compared to FA exposed mice. The source of Ly6chi monocytes in lung appeared to be from a hematopoietic source (likely bone marrow) as evidenced by more Ly6chi monocytes in blood and a corresponding decrease in the bone marrow (blood, 3.2-fold increase $p<0.05$ and bone marrow, 1.2-fold decrease, PM2.5 *vs* FA). AMΦ ($CD64^{+}CD11c^{hi}CD11b^{lo}$) in both $PM_{2.5}$ and FA mice were primarily CD45.2 at 4 weeks with no evidence of hematopoietic contribution. In contrast at 32 weeks, CD45.1 cells constituted 43.9±18.2% of AMΦ with evidence of enhanced apoptosis (Annexin V^{+}) and decreased proliferation ($BrdU^{+}$) in CD45.2 AMΦ. RNA-seq analysis of CD45.1 inflammatory AMΦ in the $PM_{2.5}$ exposed mice was consistent with a unique inflammatory transcriptomic signature signifying a $PM_{2.5}$ effect. $PM_{2.5}$ entrains a systemic bone marrow-derived CD45.1 response that together with enhanced apoptosis of tissue-resident CD45.2 AMΦ likely contributes to disrupted pulmonary immune homeostasis and perpetuation of chronic inflammation.

1140 Diesel Exhaust Particles Induce Autophagy in Bronchial Epithelial Cells

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Exposure to diesel exhaust particles (DEP) can exacerbate asthma by inducing oxidative stress, inflammation, and cellular injury in the lung. Recent studies have reported increased levels of autophagy markers in asthmatic individuals. In addition, polymorphisms in autophagy-related genes have been associated with a reduced lung function in individuals with moderate to severe asthma. Since autophagy can be induced by oxidative stress, we hypothesize that DEP alters autophagy in human bronchial epithelial cells. To determine DEP effects in the autophagy process, primary bronchial epithelial cells derived from asthmatic (DHBE) and non-asthmatic (NHBE) individuals were exposed to physiologically relevant concentrations of DEP (5-50 µg/mL). The amount of autophagic vacuoles were determined using a fluorescent dye and time-response experiments were conducted to assess autophagy flux. Preliminary results showed that in DHBE cells, DEP induced autophagy in a dose-dependent manner. Meanwhile, in NHBE cells an increase was only observed at the highest concentration (50µg/mL). The increase in autophagic vacuoles was not due to inhibition of the autophagy flux as DEP did not have an effect in p62 degradation rate. Further experiments will be conducted to evaluate if DEP induces the phagophore formation. Our results suggest that DEP can alter the autophagy process in bronchial epithelial cells. In addition, that asthmatic cells can be up to 10 times more susceptible to DEP when compared with normal bronchial epithelial cells.

1141 Beyond the Barrier: Transepithelial Exposures Reveal Novel Roles for Oxidative Stress and Proinflammation within the Airway Microenvironment

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Oxidative stress and the release of pro-inflammatory mediators are well established events following inhaled chemical exposure; however, our understanding of the cellular/molecular pathways involved in inhalation toxicity within the airway microenvironment (AME) remain limited due to studies centered around a single airway cell type (*i.e.,* epithelial cells), and lack of physiologically-relevant studies on cell types beyond the epithelial barrier. Utilizing an organotypic model that recapitulates the "trans-epithelial" nature of analogous exposures on *in vivo*, we investigated the effect of diesel exhaust particulates (DEP) on human bronchial epithelial cell (HBEC) and human lung fibroblast (HLF) signaling within the AME. We hypothesized that trans-epithelial DEP (TE-DEP) exposure mediates oxidative stress/pro-inflammatory response through an imbalance in oxidant/antioxidant gene expression and alterations in key cellular stress-responsive pathways. Live-cell imaging with a redox-sensitive fluorescent biosensor (pLV-roGFP-HyPer Red) defined the dynamics of glutathione oxidation and H_2O_2 signaling in HLF, which peaked at 6 hours following TE-DEP exposure. Likewise, the oxidative stress-responsive gene, HMOX-1, peaked at 6 hours in both HBEC and HLF, while the pro-inflammatory gene, IL-8, peaked at 4 hours in HBEC and 10 hours in HLF, by which time HMOX-1 returned to baseline. Oxidative stress arrays identified additional genes (*i.e.,* NQO1, PTGS2, and GCLM1) induced following TE-DEP exposure. Pretreatment of HLF with free radical scavengers reduced DEP-dependent HMOX-1 and IL-8 expression in HLF and adjacent HBEC. Temporal analysis of stress-responsive MAPK, NF-kB, and NRF2 pathways confirmed DEP-mediated and time-dependent phosphorylation of key cellular targets, and pretreatment with MAPK inhibitors attenuated DEP-dependent HMOX-1 and IL-8 expression. Further, neutrophil chemotaxis increased following exposure to TE-DEP conditioned medium indicating DEP-mediated changes in recruitment. This study is the first to characterize the dynamics of oxidative stress/pro-inflammatory signaling following trans-epithelial chemical exposure, providing novel insight for the development of therapeutic interventions to reduce adverse effects of inhaled chemical exposure. *Does not reflect Agency policy.*

1142 Rodent and Human TRPA1, V1, and V3 Exhibit Different Responses to Prototypical and Particulate Matter Agonists

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People are exposed to numerous forms of particulate matter (PM) in the course of daily activities. Exposures can contain coal fly ash (CFA), diesel exhaust particles (DEP), cigarette smoke PM (CSPM), calcium oxide (CaO), wood smoke (WSPM), and more. In the lung, activation of transient receptor potential (TRP) channels are one mechanism by which PM can trigger adverse effects. Human TRPA1 is activated by CFA, DEP and WSPM, as well as the soluble agonist allyl isothiocyanate (AITC). Human TRPV1 is activated by the soluble agonist nonivamide and CFA and TRPV3 is activated by the soluble agonist drofenine and WSPM. We have observed that responses of TRP channels to particles differ across species, particularly rodents and humans. This variability could have implications for studies attempting to translate results from mice to humans and vice versa. Alignment of human and mouse TRPA1, V1 and V3 amino acid sequences highlight differences in the pore-loop regions of these TRP channels which may partially explain species differences in the response to various PM. Mouse and human TRPA1, V1 and V3 plasmids and mutants thereof, were transiently transfected into GcAMP6-over-expressing HEK-293 reporter cells and calcium flux assays were performed to examine the basis for species-specific differences in responses to PM and known soluble agonists. For mouse TrpV3 the response to WSPM was decreased compared to human and this decrease involved the amino acids P612 and N616 in the pore-loop region. Mouse TrpV1 also had reduced responses to CFA and CaO compared to human TRPV1. Key amino acids were D605, S609 and P619 in the pore-loop region while S609 and P619 dictated the response to CaO. However, mouse TrpA1 exhibited an increased response to DEP, CSPM, WSPM, and CFA when compared to human TRPA1. Studies are ongoing to ascertain if pore-loop residues of TRPA1 underlie this effect. Identification of specific PM-sensing sites on TRP channels furthers our knowledge of the mechanisms by which TRPs are activated by PM and reveal potential limitations in animal models to study TRP channel contributions to particle toxicities in humans. *Support: ES017431, ES027015.*

1143 Interactions among the Particulate Matter (PM) Components for Cytotoxicity

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Several studies have shown the associations of ambient particulate matter (PM) concentrations with many adverse health effects. The toxic effect of PM is related to its chemical composition, and many hazardous components in PM have been spotted. However, the toxicity contribution from their interaction can not be neglected. To understand the interaction, in this study, we have chosen three metals (Cu, Fe, and Mn) and four quinones (9,10-phenanthraquinone, 1,2-naphthoquinone, 1,4-naphthoquinone, and 5- hydroxy-1,4-naphthoquinone), that are most abundant in PM, in addition to the ambient humic-like substances (HULIS) from the real PM samples. We exposed the Chinese hamster ovary (CHO) cells to the mixtures of these components for 72 hours to assess the cytotoxicity and obtained the lethal concentration that causes 50% inhibition (LC50). Mixture toxicity index (MTI) method was used to determine the interaction. The MTI result indicates that Fe is synergistic with most quinones and HULIS in cytotoxicity. The interactions among quinones are mostly additive. Our results reveal that metals and organic compounds in the environmentally relevant ratio are not simply additive; they are probably synergistic in most circumstances.

1144 Single Cell RNA Sequencing Reveals a Unique Monocyte Population in Bronchoalveolar Lavage Cells of Mice Challenged with Afghanistan Particulate Matter and Allergen

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Upon returning from deployment to Afghanistan, increasing numbers of service personnel are reporting symptoms consistent with an asthma-like airways disease including coughing, wheezing, and airway hyperresponsiveness. It is thought that prolonged inhalation of toxic desert silicate-containing particulate matter (PM) may contribute to this deployment-related lung disease (DRLD). Furthermore, symptoms of DLRD persist in the post deployment setting, where soldiers are exposed to aeroallergens such as house dust mite. The goal of this study was to define the transcriptomic responses of each type of leukocyte found in the airway lumen and airspace after inhalation of Afghanistan desert particulate matter (APM) and subsequent challenge to house dust mite (HDM) in mice. C57BL/6 mice (n=15/group) were exposed to filtered air or aerosolized APM for 6 hours per day, 12 consecutive days using a whole body exposure system, followed by intranasal PBS or HDM allergen challenges to mimic asthma. One day after the last allergen challenge, mice were euthanized to collect bronchoalveolar lavage cells for assessment of inflammation and single cell RNA sequencing (scRNAseq) using the 10x Genomics Platform. The flexiVent small animal respiratory physiology system was used to measure airway hyperresponsiveness (AHR) using total respiratory system resistance (Rrs). APM exposure followed by allergen challenge trended to increase AHR compared to filtered air controls (average Max(Rrs) 1.4 or 2.9 cmH2O.s/mL, respectively). In mice exposed to APM, allergen challenge significantly increased inflammation (total leukocyte count: p=0.01, absolute eosinophil numbers: p=0.01). Unsupervised clustering of BAL cell scRNAseq data revealed a unique monocyte population induced only by both APM and allergen treatments. This population of monocytes is characterized by a gene expression signature of 606 genes, many of which belong to pathways involved in worsening of asthma-like airways diseases, such as Alox15, which is involved in airway obstruction; Itgam, which mediates inflammation via regulating leukocyte adhesion/migration; and Ccl24, which induces recruitment of eosinophils. These results demonstrate that exposure to APM while on deployment to Afghanistan can prime airways to be more responsive to allergen exposure after deployment, and that the underlying mechanisms may involve induction of a unique lung monocyte population that has the ability to regulate AHR and inflammation. *Funding by DOD grant (W81XWH-16-2-0018).*

1145 Ambient Particulate Matter-Induced Cardiotoxicity and Pulmonotoxicity in C57/B6 Mouse

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Ambient particulate matter exposure is associated with elevated cardiovascular risk in general population. To evaluate the potential cardiotoxicity following particulate matter (PM) exposure, an individual ventilated cage (IVC) system is utilized to expose C57/B6 mouse to either ambient air with particulate matter pollution (From 2018-2019 winter, Shijiazhuang, China), or filtered clean air for 21 or 42 days. To explore the potential molecular mechanism, WY 14,643 and GW6471, agonist and antagonist for peroxisome proliferator-activated receptor alpha (PPARa), were co-administered to the animals. At desired time points, the heart and lung tissues were collected, and potential morphological and functional changes following the PM exposure were assessed with histopathology and echocardiography, respectively. Additionally, immunohistochemistry for alpha-smooth muscle actin (SMA) was used to assess the fibrotic changes and cardiopulmonary effects in the lungs. The results indicated that right ventricular wall thickness was increased and the heart rate was elevated following PM exposure, while increased fibrotic changes were indicated by Masson trichrome staining and alpha-SMA immunohistochemistry. Alpha-SMA immunohistochemistry also indicated that the smooth muscle layer of small pulmonary arteries thickened following PM exposure, suggesting that the changes in right ventricle is secondary to the small pulmonary arteries. Interestingly, co-treatment with PPARa agonist WY 14,643 alleviated such changes, while GW6471 either had little effects or exacerbated such changes, suggesting that interference with PPARa signaling and fatty acid metabolism is contributing to the endpoints observed in this study. In summary, exposure to ambient PM resulted in morphological and functional changes in the hearts, fibrotic changes and thickened smooth muscle layer in small pulmonary arteries in the lung of C57/B6 mouse, in which interference with PPARa signaling is involved. *This work was supported by National Natural Science Foundation of China (Grant No.81872591, 91643203, 81502835).*

1146 Exposure to Airborne Fine Particulate Matter Is Associated with Impaired Endothelial Function and Biomarkers of Oxidative Stress and Inflammation

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Epidemiological evidence suggests that exposure to air pollution is associated with cardiovascular disease (CVD). While ambient air pollution is a complex mixture of particulate matter (PM), gases and metals, the association between PM exposure and CVD is particularly strong. However, the pathophysiologic mechanisms underlying this association are not completely understood. Thus, we conducted a cross-sectional study of 100 participants, recruited from the University of Louisville Hospital Clinics. Peripheral endothelial function in these participants was assessed by calculating a reactive hyperemia index (RHI). Urine samples were used to measure isoprostanes, while plasma levels of cytokines, adhesion molecules, and proteases were measured using a Luminex-based assay. Ambient levels of fine PM ($PM_{2.5}$) were obtained by calculating the daily average of all regional EPA-validated monitoring stations in the Louisville, KY region. Adjusted associations between $PM_{2.5}$ levels and measured outcomes were tested using generalized linear models. We found that there was a 12.4% decrease in RHI with every 10μg/m^3 $PM_{2.5}$ increase (95% CI: -21.0, -2.7). F-2 isoprostane metabolite, a measure of oxidative stress, showed a positive association of 28.4% per 10μg/m^3 $PM_{2.5}$ (95% CI: 2.7, 60.3). We also observed positive associations with angiopoietin 1 (17.4%; 95% CI: 2.8, 33.8), vascular endothelial growth factor (10.4%; 95% CI: 0.6, 21.0), placental growth factor (31.7%; 95% CI: 12.2, 54.5), intracellular adhesion molecule-1 (24.6%; 95% CI: 1.6, 52.8), and matrix metalloproteinase-9 (30.3%; 95% CI: 8.0, 57.5) per 10μg/m^3 increase in $PM_{2.5}$. Additionally, we observed a negative association between $PM_{2.5}$ and vascular cell adhesion molecule-1 of -15.9% per 10μg/m^3 increase (95% CI: -28.3, -1.3). Thus, exposure to $PM_{2.5}$ was associated with impaired vascular function, which may result from oxidative stress and inflammation, thereby leading to a pro-atherogenic state.

1147 Cytotoxicity of Low Concentrations of Ultrafine Diesel Exhaust Particles on Endothelial and Microglial Cell Monocultures and Mixed Co-cultures

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Diesel exhaust particles (DEPs) are a recognized risk factor for several health conditions including neurodegenerative diseases. However, information regarding the toxicity of low concentrations of ultrafine (UF) DEPs in the brain is limited. While *in vitro* studies of cerebral capillary endothelial cell (CEC) monocultures provide insight into the cellular mechanisms by which particulate matter (PM) cause toxicity, they do not account for mitigating or aggravating effects of cell-cell interactions on PM toxicity. The goals of this study were to evaluate the cytotoxicity of low concentrations of UF DEPs and to investigate if cell-cell interactions mitigate or aggravate the effects of UF DEP exposure. Rat brain microvascular endothelial cells (BMVECs) were used as a simple blood-brain-barrier (BBB) model, and rat microglia (primary and Highly Aggressive Proliferating Immortalized) as model immune cells of the brain. To determine cytotoxicity of UF-DEP and investigate the effect of cell-cell interactions, this study measured LDH leakage, cell metabolic activity (CMA), and ROS production in five different *in vitro* cell culture systems: endothelial (E), microglial (M), and HAPI (H) monocultures, and endothelial-microglial (EM) and endothelial-HAPI (EH) mixed co-cultures. The cells were exposed to 2 ng/ml, 1 µg/ml, and 20 µg/ml UF DEPs in 96-well plates for 24 hours before assaying. Results indicated that low concentrations of UF DEPs significantly decreased cell viability (i.e. increased LDH leakage and decreased cell metabolic activity) and increased ROS production in a dose-dependent manner in most cell culture systems but differentially across systems. Additionally, while the co-cultures responded differently from one another, cell-cell interactions in the EM co-culture slightly but significantly aggravated the LDH leakage and ROS production, but did not decrease CMA, compared to the E monoculture. This study demonstrated that exposure to low concentrations of UFDEPs can disrupt the function of CECs and microglia, and that cell-cell interactions can influence the degree of cytotoxic effects from exposure to environmental toxicants. Therefore, better models that recapitulate the complete neurovascular unit and its complex intercellular interactions *in vitro* should be employed when studying toxicity and environmental exposures in the BBB.

1148 AMPK Activation Attenuates Inflammatory Response to Reduce Ambient $PM_{2.5}$-Induced Metabolic Disorders in Healthy and Diabetic Mice

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Epidemiological and experimental studies have indicated that ambient fine particulate matter ($PM_{2.5}$) exposure is associated with the occurrence and development of metabolic disorders such as obesity and type 2 diabetesmellitus (T2DM). However, the mechanism is not clear yet, and there are few studies to explore the possible prevention measure. In this study, C57BL/6 and db/db mice were exposed to concentrated $PM_{2.5}$ or filtered air using Shanghai-METAS for 12 weeks. From week 11, some of the mice were assigned to receive a subcutaneous injection of AMPK activator (AICAR).The results showed that $PM_{2.5}$ exposure induced the impairments of glucose tolerance, insulin resistance, lipid metabolism disorders and disturbances of energy metabolism in both C57BL/6 and db/db mice. These impairments might be consistent with the increased respiratory, circulating and visceral adipose tissue (VAT) inflammatory response, which was characterized by the release of IL-6 and TNF-α in lung, serum and VAT. More importantly, AICAR administration led to the significant enhancement of energy metabolism, elevation of AMPK as well as the decreased IL-6 and TNF-α in VAT of $PM_{2.5}$-exposed mice, which suggesting that AMPK activation might attenuate the inflammatory responses in VAT via the inhibition of MAPKs and NFκB. The study indicated that exposure to ambient $PM_{2.5}$ under the concentration which is often seen in some developing countries could induce the occurrence of metabolic disorders in normal healthy mice and exacerbate metabolic disorders in diabetic mice. The adverse impacts of $PM_{2.5}$ on insulin sensitivity, energy homeostasis, lipid metabolism and inflammatory response were associated with AMPK inhibition. AMPK activation might inhibit $PM_{2.5}$-induced metabolic disorders via inhibition of inflammatory cytokines release. These findings suggested that AMPK activation is a potential therapy to prevent some of the metabolic disorders attributable to air pollution exposure.

1149 Metabolomics Approaches to Identify Plasmatic Factors Responsible for Fine Particulate Matter ($PM_{2.5}$)-Induced Vascular Insulin Resistance and Inflammation

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Exposure to fine particulate matter ($PM_{2.5}$) increases the risk of developing cardiovascular disease (CVD). Our previous studies demonstrated that exposure to concentrated $PM_{2.5}$ (CAP) induces vascular inflammation and insulin resistance in mice, which is, at least in part, dependent on pulmonary oxidative stress. Although we could attribute the vascular effects to pulmonary oxidative stress, it is unclear how oxidative stress is transmitted from the lung to blood vessels. Because research suggests that plasmatic factors that diffuse from the lung trigger exposure-induced injury in peripheral tissues, we tested whether *ex vivo* treatment of naïve aortas with plasma isolated from mice exposed for 9 days to CAP induces insulin resistance. We found that plasma from CAP-exposed mice induces insulin resistance in the isolated blood vessel, indicating that a plasmatic factor could be responsible for $PM_{2.5}$-induced vascular defects. To identify putative plasmatic factors, we investigated whether $PM_{2.5}$ exposure affects the plasma metabolome and whether the metabolite changes are dependent on pulmonary oxidative stress. For this, we exposed mice overexpressing lung-specific extracellular superoxide dismutase (ecSOD-Tg) and their wild-type (WT) littermates to CAP or HEPA-filtered air and analyzed metabolite abundances in plasma using ultra-performance liquid chromatography mass spectrometry (UPLC-MS). Metabolomics showed that CAP exposure impacted the level of 93 metabolites ($p \leq 0.05$, out of 724 compounds) in WT mice while in ecSOD-Tg mice only 24 metabolites changed significantly. Metabolites that increased in plasma of CAP-exposed WT mice were saturated and unsaturated fatty acids as well as inflammatory mono- and di-hydroxy fatty acids. In additional studies, we found that fatty acids such as palmitate are sufficient to induce inflammation in naïve aortas and HUVEC cells. Additionally, we found that CAP exposure decreased circulating phosphatidylcholines and phosphatidylethanolamines and increased circulating glycerol in WT mice. Our findings suggest that pulmonary oxidative stress caused by $PM_{2.5}$ exposure increases circulating fatty acids, which trigger vascular insulin resistance and inflammation that could contribute to an increased risk for developing CVD.

1150 The Cardiovascular Effects of Ovarian Hormone Removal in apoE-/- Mice Chronically Exposed to Concentrated Ambient $PM_{2.5}$

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Post-menopausal women and women with reduced ovarian function are at elevated risk of cardiovascular disease. Particulate matter (PM) air pollution has been associated with increased incidences of cardiovascular disease leading to increased daily mortality and hospital admissions. Fine PM (Dp < 2.5 µm) contains a mixture of metallic elements, road dust, and includes ultrafine PM (Dp< 0.1 µm) encompassing vehicle emissions which, due to their large surface area/unit mass, allows for greater adsorption of reactive organic molecules and increased availability for interaction with potential cellular targets. The objective of this study was to determine whether ovariectomized (OVX), apoE-/- mice are more susceptible to the cardiovascular effects of concentrated ambient $PM_{2.5}$ (CAPs) exposure than apoE-/- mice with intact ovaries. Groups of animals were exposed to either purified air (PA) or to concentrated ambient $PM_{2.5}$ (CAPs). Mice were exposed 5 hours/day, 4 days/week for 12 weeks at the University of California, Irvine, about 1600m southwest of a major freeway. Blood pressure was measured weekly while implanted cardiac transducers continuously monitored ECGs from the mice. ECGs were analyzed at specific post-exposure times to detect change from the baseline measurements at numerous waveform parameters. OVX mice exposed to CAPs exhibited immediate shortened P-wave duration compared to intact mice exposed to CAPs which may indicate a decreased rate of electrical conduction through the atria. These OVX animals also showed a non-significant trend toward PR-interval elongation which may indicate the presence of atrioventricular blockages in the heart. The most pronounced changes to baseline ECG measurements were observed in the ST-segment. Exposed OVX animals had decreased ST-segment levels compared to the intact exposed animals. These OVX animals also exhibited a more pronounced T-wave, as measured by increased T-wave area and T-wave amplitude. An initial decrease in diastolic blood pressure in exposed OVX mice compared to controls was observed. The exposed mice also exhibited a non-significant trend towards decreased breathing rates possibly indicating that the inhaled PM is resulting in short, shallow breaths. Hypotension can result from conditions such as heart

disease and bradycardia while S-T segment changes are common measures of myocardial dysfunction in humans which possibly lead to life-threatening cardiac events. Further investigation into the observed changes need be performed to explicate the sex-specific mechanisms corresponding to changes resulting from CAPs exposures.

1151 Identifying the Molecular Mechanisms of Air Pollution-Induced Thrombosis

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An estimated 3.5-million people die annually from air pollution-induced cardiovascular disease (API-CVD). API-thrombosis (API-T) is a main contributor to these mortalities and morbidities; however, the molecular mechanisms driving API-T are unclear. To identify the mechanisms driving API-T, we developed a tri-culture *in vitro* model that represents the interface of the respiratory and cardiovascular system, the alveolar capillary region (ACR). This organotypic model includes human alveolar-like epithelial cells (H441), human lung fibroblasts, and human lung microvascular endothelial cells (HULEC). We hypothesized that air pollutant exposure of the H441 cells would induce endothelial dysfunction in the HULEC, initiating the onset of pro-thrombotic/pro-coagulative state. To test this, we exposed confluent monolayers of H441 cells to the ubiquitous air pollutant, diesel exhaust particulates (DEP), and investigated the effect of this trans-epithelial exposure (TE-DEP) on the underlying HULEC. Upon TE-DEP exposure we identified induction of anti-oxidants such as heme oxygenase 1 (HMOX-1) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) in the HULEC. Increased nuclear factor erythoid-2 related factor (NRF2) protein and reduced glutathione redox potential were also identified in the HULEC. Concurrently, we observed decreased expression of the endothelial fibrinolytic and anti-coagulant genes, tissue-type plasminogen activator (PLAT), plasminogen activator, urokinase (PLAU), and thrombomodulin (THBD) in the HULEC, and increased expression of the procoagulant gene, coagulation factor III (F3). Increased fibrin clot formation was also observed on the HULEC post TE-DEP exposure. Lastly, F3 expression increased in primary human aortic endothelial cells upon treatment with conditioned media from the TE-DEP exposed HULEC. Collectively, these data suggest that TE-DEP exposure induces redox dysfunction and endothelial pro-thrombotic/pro-coagulative activation in the ACR, and suggest that ACR-secreted factors may initiate a systemic pro-thrombotic/pro-coagulative state upon TE-DEP exposure. We conclude that redox dysfunction and pro-thrombotic/pro-coagulative activation in the capillary beds of the ACR may be critical initiation steps in the onset of API-T. Ultimately, data from this model can be used to develop intervention strategies and identify exposure biomarkers to help prevent the onset of API-T and API-CVD.

1152 Air Pollution ($PM_{2.5}$) Triggers Insulin Resistance in Mice: Implication of Impaired Transcriptional Level of Circadian Gene and Glucose Metabolism in Brown Adipose Tissue

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Sponsor: D. Conklin

Air pollution exposure is associated with an increased risk of acute and chronic metabolic distress often leading to cardiovascular mortality. Our previous research demonstrated that $PM_{2.5}$ causes insulin resistance (IR) with onset of type 2 diabetes in a mouse model of chronic ambient air pollution exposure. However, the mechanism of $PM_{2.5}$ mediated disruption of metabolic homeostasis remains unknown. An unbiased transcriptomic analysis of $PM_{2.5}$ exposed mice revealed differential regulation of circadian genes in peripheral tissues compared to control mice exposed to filtered air (FA). Our goal is to study $PM_{2.5}$ modulation of circadian rhythm genes in brown adipose tissue that is associated with IR and cardiovascular events. We evaluated increased glucose tolerance ($p<0.0004$) and reduced energy expenditure ($p<0.001$) in $PM_{2.5}$ mice compared to FA. $PM_{2.5}$ mice exhibited increased plasma corticosterone (2258±140 vs 3795±286, $p<0.002$) and urine corticosterone levels (14.15±0.73 vs 18.26± 1.1, $P<0.01$), suggesting dysregulation of the hypothalamus-pituitary-adrenal system leading to insulin resistance. These results corroborated with reduced FDG uptake in the brown adipose tissue due to the reduced prdm16 and UCP 1 mRNA expression. Interestingly, the same tissues exhibited a reduced expression of circadian genes Bmal1, Clock, Cry1 and Cry2 in $PM_{2.5}$ mice as validated by qPCR. Our study suggests a previously unrecognized role for $PM_{2.5}$ as a circadian rhythm disruptor that may act as a critical regulator of glucose homeostasis in the pathogenesis of insulin resistance.

1153 Air Pollution Exposure as a Circadian Disruptor: Implications for Insulin Resistance and Type 2 Diabetes

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Environmental stressors like air pollution represent an increasingly recognized cause of non-communicable disease with recent estimates implicating 9.1 million deaths globally. Particulate matter <2.5 μm ($PM_{2.5}$) induced insulin resistance (IR) and type 2 diabetes, may represent a proximate mechanism, but common integrative mechanisms are currently lacking. In an initial unbiased transcriptomic analysis on the livers of $PM_{2.5}$ exposed mice, circadian genes were the most common gene ontology (GO) terms in response to exposure. C57BL6 male mice were exposed to 24-weeks of FA/$PM_{2.5}$ and simultaneously exposed to dim light at night (LAN) or dark at night (DAN). Metabolic cage experiments showed that compared with DAN FA, $PM_{2.5}$ exposed mice had significantly reduced energy expenditure, respiratory quotient, and VO_2 consumption, indicative of metabolic dysfunction. LAN exposure mimicked IR and glucose intolerance phenotype observed with $PM_{2.5}$. Validation of transcriptomic data by qPCR analyses revealed that $PM_{2.5}$ and LAN mice regulated expression of BMAL1, CLOCK, Per1, Per 2, Cry1 and Cry2. When compared with FA/DAN mice, the oscillatory phase and amplitude of circadian genes were altered by $PM_{2.5}$ and comparable to LAN exposure. Chronic $PM_{2.5}$ exposure and LAN, instigates insulin resistance and dysregulation of core components of liver circadian genes, raising the possibility that $PM_{2.5}$-induced IR is mediated by circadian disruption of core clock genes in liver. The role of other peripheral and central circadian genes in the pathogenesis of $PM_{2.5}$-mediated IR and its causal pathways deserve further investigation.

1154 Fine Particulate Matter ($PM_{2.5}$) Exposure Induces Vascular Circadian Misalignment without Affecting the Central Clock

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Exposure to fine particulate matter ($PM_{2.5}$) increase the risk for cardiovascular disease and diabetes. In mice, exposure to concentrated $PM_{2.5}$ (CAP) exacerbates diet-induced systemic insulin resistance, and diet-independently induces vascular inflammation and insulin resistance. However, it remains unclear how $PM_{2.5}$ triggers inflammation and suppresses insulin signaling in the blood vessel. Because inflammation and insulin resistance are associated with the disruption of peripheral circadian clocks in the vasculature we examined the effects of $PM_{2.5}$ on the circadian oscillation of vascular core clock genes. For this, mice maintained under controlled light conditions [12h light (>200 lux), 12h dark (<10 lux)] were exposed for 30 days (6h/d) to CAP or HEPA-filtered air. Aortas were collected at Zeitgeber times (ZT) 3, 7, 11, 15, 19, and 23 to analyze the 24h circadian gene expression by *RT*-PCR. To examine central effects, functional circadian pattern (e.g., activity) were measured using metabolic cages. Circadian pattern of the expression of core clock (e.g., *circadian-locomotor-output-cycle-kaput, clock* and *cryptochrome2, cry2*), antioxidant defense (e.g., *extracellular superoxide dismutase*, *sod3* and *nuclear factor (erythroid-derived 2)-like 2, nrf2*) and proinflammatory (e.g., *interleukin1β*, *il-1β*) genes were analyzed using the cosinor method that follows changes in acrophase, amplitude, and estimating statistic of rhythm (MESOR). We found that while aortic expression of *clock* and *sod3* show significant rhythmicity (*clock*: p=0.02, *sod3*: p= 0.01, n=5) in air-exposed mice, no rhythmicity (*clock*: p=0.91, *sod3*: p= 0.37, n=5) was found in CAP-exposed mice indicating the loss of 24 h circadian rhythmicity of the peripheral circadian clock due to CAP exposure. CAP exposure also changed amplitude (*cry2*) and period (*il-1β*) or induced a shift in phase (*nrf2*). While $PM_{2.5}$ exposure disrupts vascular circadian expression of core clock, antioxidant and inflammatory genes, no CAP-induced central changes (e.g., activity) or effects on insulin resistance and obesity were observed. Our data suggest that $PM_{2.5}$ exposure disrupts peripheral vascular clocks without affecting central clock regulation. CAP-induced vascular circadian misalignment could trigger vascular inflammation and insulin resistance that may be an important mechanism underlying $PM_{2.5}$-induced vascular injury that could accelerate progression to systemic insulin resistance.

1155 Exposure to $PM_{2.5}$ from Kaohusing, Taiwan, Induces Prenatal Oxidative Stress and Fetal Brain Developmental Alternation in the Sprague-Dawley Rat Model

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Epidemiologies suggest that an increase of $PM_{2.5}$ in ambient air corresponds to an increase in lung and cardiovascular diseases. Recent studies show that $PM_{2.5}$ travels down the respiratory tract, into the lungs, enters systemic blood circulation, and increases the incidence of respiratory diseases among newborn infants which might cause chronic inflammation in pregnant women and retard fetal development. Our study indicated that intratracheally exposed prenatal rats to $PM_{2.5}$ collected from Kaohusing, Taiwan might cause dose-independently decrease both of pregnancy rate and birth rate, and induce cytokines and free radicals release in the amniotic fluid of the pregnant. On E15~E17, pregnant SD rats were injected with Bromodeoxyuridine (BrdU) (s.c.) to detect proliferating cells, and the rats were euthanized on E18 an hour after the last $PM_{2.5}$ exposure. Blood and tissue samples were collected, the results showed no significant damage of $PM_{2.5}$ to the maternal organs. Nevertheless, the maternal body weight of rats exposed to $PM_{2.5}$ experienced a constant decline, with increasing white blood cell count and an elevated inflammatory cytokine in the blood. Furthermore, pregnant rats exposed to $PM_{2.5}$ had a noticeable higher level of ROS in the amniotic fluid as well as a higher number of stillbirths. In the fetal brain, results have shown that the total amount of glutathione (GSH) were significantly lower, with an increase level of inflammatory cytokine. On the other hand, the fetal cerebral cortex on E18 were collected, and we found that laminar positioning of early born cortical cells expressing SATB2 and CTIP2 were disturbed, with a scattering distribution. Thus, we demonstrated that exposure of $PM_{2.5}$ to pregnant rats might lead to an increased inflammatory response and integrate into pre-existing neuronal circuits.

1156 Differential Effects of Ultrafine, Fine, and Coarse PM from Imperial Valley, California

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Particulate matter (PM) in Imperial Valley, California comes from a variety of sources including, but not limited to: agriculture, feedlots, vehicles at the border crossing, the megacity of Mexicali, and the dry lakebed of the evaporating Salton Sea. To determine if there are differences in the composition and biological response to Imperial County PM by size, ambient PM samples were collected from a sampling unit stationed just south of the Salton Sea. Ultrafine ($PM_{0.1}$), fine ($PM_{2.5}$) and coarse (PM_{10}) samples were collected and extracted separately. Analyses of the chemical compositions were performed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and high-resolution time-of-flight aerosol mass spectrometry (HRAMS). Endotoxin and ß-glucan levels were also quantified using *Limulus* Amebocyte Lysate (LAL) pathway assays. An aliquot of each sample was heated overnight to remove any bioaerosols present. Biological response was measured by exposing a cell line of macrophages (U937) to PM for 24-hours and quantifying the response with quantitative real-time Reverse Transcription-Polymerase Chain Reaction (qPCR). The analyses of the chemical characterizations by ICP-MS and HRAMS did not demonstrate observable differences between particle sizes. More analysis on the organic compounds present in each sample is required. Biological measurements demonstrate that PM_{10} induces an inflammatory response in macrophages, with significant increases in the gene expression of *IL-6, IL-8, COX-2* and *CXCL1*. $PM_{0.1}$ demonstrates a significant increase in expression of *CYP1a1*. Heat-treatment of PM samples significantly lowered the levels of endotoxin and ß -glucans in $PM_{0.1}$ and $PM_{2.5}$, but not in PM_{10}. However, the biological response to PM_{10} significantly changed before and after heat-treatment, suggesting an additional chemical change unrelated to endotoxin or ß -glucan activity. This difference in response was not observed in heat-treated $PM_{0.1}$ or $PM_{2.5}$. Residents in Imperial Valley have access to 24-hour air quality data from 50 different monitors that give real-time levels of $PM_{2.5}$ and PM_{10} and also provide a recommendation on healthy practices dependent on the current AQI (air quality index). While these results are useful for daily considerations, there is no information on the composition or toxicity of ambient PM. In contrast, the cell-screening methodology described can provide definitive information on the toxicity of PM and further inform community members on the quality of local air.

1157 Cache Valley $PM_{2.5}$ Activates Akt, Inflammatory Pathways, and Induces Genetic Damage in Human Lung Cells

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Northern Utah's Cache Valley frequently has some of the nation's highest concentrations of $PM_{2.5}$ particulate air pollution (CVPM). Exposure to $PM_{2.5}$ is associated with increased all-cause mortality, cardiovascular and cardiopulmonary diseases, heart attack, stroke, COPD, Alzheimer's disease, and lung cancer. The purpose of this study was to determine the cellular responses of human lung cells (BEAS-2B) exposed to CVPM (1 and 12 µg/ml; 24 hr) collected onto stainless steel plates using a Tisch impactor to minimize particle extraction artifacts associated with filter-based collection. Parallel experiments were conducted with diesel exhaust particles (DEP) as a positive control. Exposure to CVPM resulted in genetic damage as assessed by the Comet Assay, with potency equivalent to that of DEP, and a significant increase in the number of actively-dividing cells compared to control as assessed by flow cytometry ($p < 0.05$). Whole-genome microarray (Affymetrix Human 2.0) identified affected genes principally related to the inflammatory and immune pathways, as well as activated serine/threonine Akt (*aka* protein kinase B or PKB)-dependent pathways, among others. Subsequent qRT-PCR showed that CVPM exposure significantly increased expression of inflammatory markers including IL-6, CD40LG, and PLAG27 as well as cytochrome P450 (CYP) 1A1. Immunoblotting confirmed activation of Akt by phosphorylation of Thr308 in both CVPM and DEP exposed cells. This data from experiments where ambient CVPM was collected onto a solid substrate agrees in with prior findings that CVPM upregulates inflammatory pathways as well as activates Akt with potency similar to that seen with DEP. In total, our data supports the hypothesis that CVPM toxicology involves pro-inflammatory and carcinogenic pathways. *This research is supported by the Marriner S. Eccles Charitable Foundation and by Utah State University.*

1158 Characterization of the Inorganic Compounds Found in Airborne $PM_{2.5}$ in Two Sites from Puerto Rico (Urban and Rural)

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The exposure to the chemical constituents of airborne particulate matter (PM) is an important factor when considering their potential health effects. Transition metals, capable of redox cycling, are known to contribute significantly to the exacerbation of respiratory ailments. These transition metals are enriched in airborne PM from polluted urban and industrial sites. Exposure to these constituents result in the induction of oxidative stress in the bronchial epithelium and furthermore, promote the secretion of inflammatory mediators. Therefore, it is important to know the contributions of $PM_{2.5}$ constituents from both rural, and urban (more exposed to industrial activity) sites in order to investigate their relationships to toxic responses due to atmospheric exposure. $PM_{2.5}$ samples from a Rural (Humacao) and an Urban site (Bayamon) in Puerto Rico were analyzed for inorganic constituents. Samples from both locations were subjected to a Microwave-Assisted digestion and the resulting extracts characterized for their inorganic constituents by means of Induced Coupled Plasma Mass Spectrometry (ICP-MS). Approximately 59 trace elements were evaluated, of which 8 are considered with the greatest toxic potential. Higher $PM_{2.5}$ concentrations were found at the urban the site (5.12 ug/m^3) compared to the rural site (4.33 ug/m^3). During summer, African Dust Events are common, reaching into the rural site and contributing to its $PM_{2.5}$ concentration. The concentration at the rural site was found to be higher during these months compared to the urban site. Average Copper and Lead concentrations were three and two-fold higher at the urban site (0.71 ng/m^3 and 0.05 ng/m^3 respectively) compared to the rural site (0.19 ng/m^3 and 0.02 ng/m^3). Of the eight potential toxic metals considered, only Iron was found to be higher at the rural site (19.88ng/m^3 compared to 21.19 ng/m^3 in the urban). These results become key elements for future $PM_{2.5}$ extract comparisons between sites after Hurricane Maria by employing cytotoxic response using bronchial epithelium as cell model of respiratory distress due to exposure.

1159 $PM_{2.5}$ from a Marcellus Shale Drilling Operation Induces Cardiovascular Toxicity

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Unconventional natural gas well development (UNGD) of the Marcellus Shale geological formation has continued to be a burgeoning energy driver in the US. Thus, rural communities and regions are experiencing increased industrial activities and air pollutant exposures. Our laboratory has identified increased concentrations of particulate matter (PM) in the fine (<2.5 μm, $PM_{2.5}$) and ultrafine (<0.1 μm, $PM_{0.1}$) size ranges near UNGD areas. Furthermore, negative consequences of gas well emissions on health outcomes have been reported in the epidemiological literature. We collected high-volume $PM_{2.5}$ samples onto PTFE filters over 1 week during fracture stimulation at a Marcellus Shale gas well site. Additional samples were taken upwind, and downwind from the well pad during the same time-frame. The samples were liberated from the filters into ultrapure water and dried via lyophilization. Previous *in vitro* data has shown significant cytotoxicity based on distance downwind from the well pad. Young Sprague Dawley rats were exposed to 100 or 300 μg/rat $PM_{2.5}$ from the drill site via intratracheal instillation. $PM_{2.5}$ significantly increased heart rate (HR, Sham 317±8 BPM vs. $PM_{2.5}$ 342±8 BPM). However, separately 100 or 300 μg/rat did not significantly alter HR. *In vivo*, arteriolar responses to metabolic vasodilation, endogenous neurotransmitter vasoconstriction, and endothelial vasodilators were negative. In isolated mesenteric arterioles, there was significant enhancement of phenylephrine-induced vasoconstriction 1 nM (% Max Constriction Sham 2.5±1.0%, $PM_{2.5}$ 8.8±2.6%). Taken together, these data suggest that exposure can significantly increase heart rate, and induce arteriolar vasoconstriction, though the mechanisms are unknown. *Funding: NIEHS R15ES028005.*

1160 Temporal Differences in Oxidative Potential and Chemical Composition of $PM_{2.5}$

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Exposure to fine particulate matter ($PM_{2.5}$) has well-established systemic human health effects. Oxidative stress is a hypothesized mechanism for the health effects associated with $PM_{2.5}$ exposures. Oxidative potential (OP) is the measure of a substance's capacity to oxidize a target molecule. The OP of $PM_{2.5}$ has recently been suggested as a measure that is more indicative of human health effects than the routinely measured $PM_{2.5}$ concentration. The purpose of this experiment is to analyze the OP of $PM_{2.5}$ collected on air filters and determine if there differences in the OP of $PM_{2.5}$ collected from the same location on different days. $PM_{2.5}$ was collected onto PTFE-coated filters from a monitor placed in a public park in Eugene, OR on different days in the Winter. $PM_{2.5}$ will be extracted from each filter via sonication in methanol. An aliquot of the extraction solution will be used to measure OP using the dithiothreitol (DTT) assay. An additional aliquot will undergo analysis via inductively coupled plasma - mass spectrometry (ICP-MS) to quantify elements (n=30). Correlations between OP, $PM_{2.5}$ mass, and chemical composition will be made. Initial testing of a subset of the filters shows significant differences in elements based on the day that $PM_{2.5}$ was collected, including: Cd, Ce, and Pb ($p \leq 0.05$, one-way ANOVA). The DTT assay has been optimized and calibration curves for the assay are reproducible with no significant difference observed between replicates and with an r^2 value consistently above 0.99. $PM_{2.5}$ from the park location collected on different days has shown up to a 2-fold difference in OP for a subset of samples (range of 8.3 to 4.2 nM DTT consumed/μg $PM_{2.5}$ for three filters). We anticipate to see differences in OP for $PM_{2.5}$ collected on different days due to the chemical composition, particularly if redox active elements are in higher concentrations. Correlations will help us identify components of $PM_{2.5}$ that may be impacting the OP more so than the total mass. This research will add to the growing evidence and justification for investigating the OP of $PM_{2.5}$.

1161 Effect of a High-Fat Diet and Occupational Exposure in Different Rat Strains on Lung and Systemic Responses: Development of an Animal Model to Examine the Exposome

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The exposome is the measure of all exposures of an individual in a lifetime and how those exposures relate to health. Important components of the exposome include lifestyle (diet), environmental and occupational exposures, and individual genetic predisposition. Mapping of the exposome could improve the understanding of disease and aid in prevention strategies and possible cures of many diseases. The goal was to develop an experimental model of the exposome by collecting biological samples during critical life stages of an exposed animal that are applicable to worker populations. Genetic contributions were assessed using strains of male rats with different genetic backgrounds [Fischer-344 (F344), Sprague-Dawley (SD), Brown-Norway (BN)] maintained on a regular (REG) or high fat (HF) diet for 24 wk. At wk 7 during diet maintenance, groups of rats from each strain were exposed to welding fume (WF; 20 mg/m^3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air until wk 12, at which time some animals were euthanized. A separate set of rats from each strain were allowed to recover from WF exposure until the end of the 24 wk period. Bronchoalveolar lavage fluid and serum were collected at 7, 12, and 24 wk to assess general health indices. Exposure to WF during maintenance on a HF diet caused specific adverse health outcomes directly after exposure as well as after a 12-wk recovery phase. Depending on the animal strain, there was evidence that WF exposure and HF diet together worsened lung toxicity and kidney function as well as altered different serum enzymes and proteins. The exposomal factors of diet, exposure, and strain were all important, depending on the health outcome measured. Exposure had the most significant influence on the pulmonary responses, whereas strain and diet were the most significant contributors regarding parameters related to extrapulmonary responses. Principal component analysis further confirmed the influence of strain on the responses measured, indicating the importance of genetic predisposition as an exposomal factor. In summary, this study showed that an animal model can be useful in the assessment of the exposome as external lifetime exposures can be easily controlled and adverse health outcomes measured.

1162 Differential Responses of Murine Alveolar Macrophages to Elongated Mineral Particles of Asbestiform versus Non-Asbestiform Varieties

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Occupational exposures to asbestiform elongated mineral particles (EMP) may lead to diffuse fibrosis, lung cancer, malignant mesothelioma and autoimmune diseases. Cleavage fragments (CF) are chemically identical to asbestiform varieties of mineral, but there is no consensus on whether to treat them as asbestos from the toxicological and regulatory standpoint. Alveolar macrophages (AM) are the first-responders to inhaled particulates, participating in clearance and activating the other resident and recruited immunocompetent cells, which has an impact on the long-term outcomes. In the current study we are addressing the question of how differences in EMP crystal growth habit (asbestiform vs. non-asbestiform) affect AM responses. MPI cells, a non-transformed line that closely mimics AM phenotype, were treated with mass-, surface area- (s.a.), and particle number- (p.n.) equivalent doses of respirable asbestiform and non-asbestiform riebeckite/tremolite EMP (median lengths 4.5-5.5 μm) for 24 h with or without LPS (5 ng/ml). We assessed viability and apoptotic response, lactate dehydrogenase (LDH) and cytokines in cell supernatants. Riebeckite/tremolite asbestos and CF were taken up and induced similar LDH leakage and decrease in viability at the s.a. equivalent doses. At the equal mass, asbestiform EMPs were clearly more cytotoxic. When treated with equal p.n., CF had more pronounced cytotoxic effects. Apoptosis induction was more pronounced in asbestos-treated cells, compared to CF in all comparisons (mass/s.a./p.n.). There was an increase in chemokines and elevated pro-inflammatory cytokine secretion compared to control. Principal component analysis of the cytokine/chemokine secretion showed close clustering for the s.a. and p.n. equivalent treatments. LPS stimulation shifted the cytokine profiles towards inflammation compared to non-LPS-stimulated cells, with more IL-1β and TNF-alpha secretion in asbestos-treated cells compared to CF. In conclusion, murine AM initial responses to respirable EMP of similar lengths, but different growth habit depend on the s.a. metric rather than the mass or the p.n. The study also confirms that asbestiform habit itself is an important determinant of some signaling pathways, i.e. apoptosis. Finding out what metric is critical for the mineral fiber toxicity is a complex task and the *in vivo* study with the same EMP is underway to further address the issue.

1163 Multimodal Mass Spectromtry Analysis following Repeated Intratracheal Instillation of Dispersed Silver Nanoparticles in Rats

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Silver nanoparticles are among the most widely manufactured nanomaterials and have been incorporated into a wide variety of consumer products such as textiles, detergents, medical devices, drug delivery products, anti-microbial

sprays, personal care products, paints/coatings, and water purification. This has led to the potential increase in risk of worker exposure to these particles. We have previously conducted an *in vivo* study to characterize pulmonary and systemic effects following repeated exposure where rats were intratracheally instilled once a week for eight weeks with 9.35 µg or 112 µg of dispersed silver nanoparticles (Nano-Ag) or dispersion medium (DM) as a vehicle control. Lung histopathology, and analyses of bronchoalveolar lavage fluid (BALF) and serum were performed at 7, 28, and 84 days after the last exposure. Lung injury was characterized by alveolar and interstitial inflammation, as well as oxidative stress. In the current study, a metabolomic approach was employed to characterized changes in the BALF and serum to further examine mechanisms of toxicity and establish a potential panel of biomarkers of exposure and effect. Metabolite characterization was conducted with matrix assisted laser desorption ionization (MALDI) and liquid chromatography mass spectrometry (LC-MS). The metabolomics analysis revealed a significant increase in 44 metabolites in BALF, including 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) and cholesterol in the rats exposed to 112 µg at the 7- and 28-day time points. Rats exposed to the high dose had a significant increase in ~60 serum metabolites, with the greatest degree of change in the alanine biosynthesis pathway intermediates in the serum, which have been shown to be indicators of liver toxicity. There was a >2.0-fold increase in alanine, valine, and glutamate in the 112 µg exposed animals at the 7- and 28-day time points. The results of this study show that pulmonary exposure to nanosilver particles leads to changes in the BALF lipidome, which may be related to the pathway of lung injury and oxidative stress. In addition, increased levels of circulating metabolites indicative of liver toxicity agree with numerous *in vivo* studies by other investigators. Further analysis of these molecules and lipids will be conducted to establish a potential biomarker panel for nanosilver exposure and effect.

1164 Single Cell RNA Sequencing and Mouse Transcriptome Analysis Show Amelioration of SEB-Induced Acute Lung Injury via Altering Metabolic Profile of THC-Treated Mice

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Inhalation of Staphylococcal Enterotoxin B (SEB) is known to induce acute lung injury (ALI) and studies from our laboratory have shown that THC, a psychoactive ingredient found in *Cannabis sativa,* can induce anti-inflammatory cells such as T regs. Lysine is an essential amino acid that plays several important roles by decreasing IFNg cytokine, inhibition of ERK1/2 and NF-κB signaling pathway, antimicrobial activity against Gram positive bacteria and production of carnitine, which is key in fatty acid metabolism and used as nutritional supplement. In the current study, we investigated the metabolic profile in ALI with or without THC treatment. Lung microbiota was collected and 16S rRNA sequencing was performed. 16S rRNA metagenomic data were generated and functional profiles predicted using PICRUST. Methionine and lysine biosynthesis were increased significantly in THC treated group while lysine degradation was upregulated significantly in vehicle group. Furthermore, we analyzed the metabolomic profile in serum by mass-spectrometry analysis and we found that lysine and carnitine concentrations were increased significantly in THC treated group. Moreover, we found by single cell RNA sequencing of whole lung tissue that solute carrier (Slc25a3 and Slc25a39) genes, which are carnitine transporters, were increased statistically in THC group in CD4+ and CD8+ T cells as well as MDSCs. In addition, carnitine palmitoyltransferase 1 (CPT1), a mitochondrial enzyme responsible for the formation of acyl carnitine which is transported from cytosol to mitochondrial matrix was also increased in CD4+ T cells and alveolar macrophages following THC treatment. Furthermore, we confirmed the results by transcriptome analysis that the solute carrier family was increased significantly in lung infiltrating mononuclear cells of THC treated group. Moreover, our studies on fuel source use showed that SEB-activated T cells treated with THC are glucose-independent while vehicle group is glucose-dependent. Together, THC modulates metabolic functions of lung microbiota and T cells which may affect their signaling, differentiation and toxicity which led to improve homeostasis in lungs. *Supported by NIH grants P01AT003961, R01AI123947, R01AI129788, R01ES019313, and P20GM103641.*

1165 Anti-TNFα Antibody Mitigates Sulfur Mustard-Induced Lung Injury in Rats

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Sulfur mustard (SM) is a vesicating chemical warfare agent that causes severe lung injury when inhaled. Acute sulfur mustard-induced toxicity is due, in part, to persistent accumulation of macrophages in the lung and the release of inflammatory mediators including cytokines, chemokines, eicosanoids and growth factors. The proinflammatory cytokine, tumor necrosis alpha (TNFα), is released from activated macrophages; it has been shown to contribute to lung injury by promoting inflammatory cell accumulation in tissues and stimulating the release of other inflammatory mediators. This leads to oxidative stress, airway hyperresponsiveness, and tissue remodeling. In these studies, we tested the hypothesis that anti-TNFα antibody treatment would mitigate mustard induced acute lung inflammation and injury. Male Wistar rats were exposed to SM vapors (0.4 mg/kg) or air control and treated with either monoclonal anti-TNFα antibody (15 mg/kg) or vehicle 15-30 min later. Animals were euthanized 3 days after exposure, bronchoalveolar lavage fluid (BAL) and lung tissue collected. Treatment of rats with SM resulted in lung injury and inflammation as measured by increases in bronchoalveolar lavage fluid (BAL) cell and protein content. SM exposure also resulted in increased numbers of lung macrophages expressing tumor necrosis factor (TNF) α and heme oxygenase (HO)-1 indicating inflammation and oxidative stress. Treatment of rats with anti-TNFα antibody (15 mg/kg, i.v.) 15-30 min after SM inhalation reduced lung injury and inflammation, SM-induced levels of HO-1 and TNFα were also suppressed by anti-TNFα antibody treatment. These data demonstrate that inhibiting TNFα may be an effective approach to mitigating acute lung injury induced by vesicants. *Supported by NIH Grants U54AR055073, R01ES004738, and P30ES005022.*

1166 Comparison of Overlooked yet Prevalent Polycyclic Aromatic Hydrocarbon Mixtures on Adverse Effects in Lung Cells

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Polycyclic aromatic hydrocarbon (PAH)s are major components of firsthand and secondhand smoke (cigarette and marijuana) and air pollution, among other sources, and thus far, research has almost exclusively focused on the higher molecular weight (HMW; >5 rings) PAHs for all health effects (e.g., asthma, inflammation and cancer). However, the low molecular weight PAHs (2-4 rings) are in higher prevalence than HMW PAHs in all exposure settings and our previous studies implicate these LMW PAHs in altering critical signaling pathways in lung cells. Specifically, we previously demonstrated that two of these LMW PAHs, 1-methylanthracene (1-MeA) and fluoranthene (Flthn), inhibit gap junctions, decrease connexin 43 (Cx43; primary connexin in lung) expression, activate MAP kinases, and induce inflammatory mediators, such as tumor necrosis factor alpha (TNF). Gap junctional intercellular communication (GJIC) is involved in lung tissue homeostasis and is reduced in early stage lung carcinogenesis. Our hypothesis is that adverse effects of PAH mixtures will differ depending on the PAHs tested. We used MTS assays for cytotoxicity, scalpel-loaded/dye transfer assays to measure GJIC, connexin (Cx)43 immunoblots, as well as quantitative RT-PCR in a mouse alveolar type II pneumocyte cell line (C10 cells) in the absence or presence of equimolar ratios of these mixtures (1-MeA:Flthn) or (1-MeA:Flthn:Phenanthrene (Phe)) at several time points. Additional studies validated findings in a human lung cell line (BEAS2B). Cytotoxicity was observed at doses >60 µM in C10 cells with both 2 and 3PAH mixtures, however, was higher in 3PAH mixtures. The 30 min dose response for GJIC showed that both PAH mixtures had more GJIC dysregulation than the individual PAHs, however, the dose response for the 3PAHs was shifted left compared to 2PAHs; similar findings were observed in the BEAS2B cells. Cx43 expression was reduced in response to either PAH mixture after 24 h. Lastly, we observed significant differences between pro-inflammatory cytokines in response to 3PAHs versus 2 PAHs. Collectively we provide evidence that a relevant environmental exposure to LMW PAHs can elicit adverse effects in lung cells and are dependent on the specific PAHs. Future studies will investigate the combination of both HMW and LMW PAHs in these responses. *Funded by R15ES024893-01 (AKB)/FAMRI CIA (AKB).*

1167 Epidemiological and Experimental Studies on a New Incident of Lung Diseases in Japanese Workers Handling Cross-Linked Water-Soluble Acrylic Acid Polymer (CWAAP) Powders

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Many workers handling CWAAP powders came down with lung diseases in a Japanese company. Since there have been no case reports on pulmonary toxicity of CWAAP, we conducted epidemiological and experimental studies to verify whether occupational inhalation exposure to CWAAP powders cause lung diseases. [Epidemiological study] The incidence of abnormal lung shadows on chest x-ray in 30 male workers (age 19-55 years) handling CWAAP powders (between September 2009 and May 2017) was compared with that of Japanese male office workers (current smokers aged 30-34 years). [Experimental study] Male Fisher 344 rats received a single intratracheal instillation (0.05-0.5 mg/rat) and a 5-day (6 hours/day) inhalation of CWAAP powders. Histopathological changes in lung tissues were examined at 3 days, 1 week, 1 month, 2 months, and 3 months. [Epidemiological study] The 3-year cumulative incidence of abnormal lung shadows was significantly higher in workers handling CWAAP powders (0.213 vs. 0.032, $p<0.001$). Chest CT scan disclosed intestinal shadows around the bronchioles predominantly in the upper fields of both lungs in all cases. [Experimental study] Infiltration of polymorphonuclear neutrophils and foamy macrophages in alveolar spaces was persistently observed at 3 days, 1 week, and 1 month in a dose-dependent manner. Interstitial collagen deposition was observed using Masson trichrome staining and immunohistochemistry for collagen type 1 at 3 months. The findings from epidemiological and experimental studies revealed that occupational inhalation exposure to high levels of CWAAP powders can induce lung fibrosis. Further studies are needed to evaluate the carcinogenetic potential of inhaled CWAAP powders. *Acknowledgement: This study was supported by the Industrial Disease Clinical Research Grant (MHLW).*

1168 Cardiopulmonary Effects of Phosphine Poisoning: A Preliminary Evaluation of Milrinone

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Phosphine gas (PH_3) is a widely used grain fumigant and common reagent in chemical synthesis. Despite commercial uses, PH_3 poses a serious threat to human health, through accidental or intentional exposure, as it is both highly toxic and easily attainable. PH_3 poisoning affects all major organ systems and is thought to specifically target oxidative respiration; nevertheless, the exact mechanism of PH_3-induced toxicity remains largely unknown. Previous studies indicate that cardiac failure and circulatory compromise may be central to PH_3-induced mortality. Here, we investigate milrinone (MLR), a phosphodiesterase-3 (PDE-3) inhibitor used to treat cardiac failure, as a potential countermeasure for PH_3 poisoning. Telemetrized female rats were exposed to PH_3 at a concentration-time product of 21450 ppm×min using a custom whole-body inhalation exposure system with integrated real-time physiological monitoring capabilities. Rats were divided into cohorts and treated prophylactically with one of three doses of MLR (40, 200, or 600 µg/kg) or water. Respiratory dynamics and cardiac parameters were recorded before, during, and after PH_3 exposure. Prophylactic administration of high dose MLR (600 µg/kg) nominally improved survivability compared to water-treated animals. However, normalized time of death did not differ substantially between different treatment groups, suggesting that MLR did not extend the treatment window for PH_3 poisoning despite reducing mortality. In terms of respiratory function, MLR did not alleviate changes in respiration and PH_3 exposure induced an increased respiratory drive regardless of treatment regimen, with observed minute volume elevations of 150 - 200%. Conversely, MLR treatment appeared to improve aspects of cardiac function affected by PH_3 exposure. In the absence of treatment, cardiac contractility (+dP/dt) increased by approximately 50% as a result of PH_3 exposure; rats administered MLR exhibited higher +dP/dt increases with slight dose dependent effects. Similarly, mean arterial pressure (MAP) increased following PH_3 exposure, however MLR treatment reduced MAP with slight dose dependent effects. These expected dose-dependent responses and increased survivability illustrate the viability of MLR as a potential countermeasure for PH_3 exposure when administered prophylactically.

1169 Single Cell RNA Sequencing Reveals Metabolic Reprogramming in Inflammatory Cells in SEB-Induced Acute Lung Injury following Treatment with Resveratrol via AhR-miR-100-Axis

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Resveratrol, (RES), a phytoalexin, is well-known for its anti-inflammatory and anti-oxidant properties. SEB, a superantigen, is known to trigger acute lung injury (ALI) and cause mortality. In the current study, we tested the effect of RES in a dual-dose model of SEB exposure that triggers ALI and causes 100% mortality in C3H/HeJ-mice. The data revealed RES attenuated SEB-induced ALI and prevented mortality. Lung-infiltrating mononuclear cells were obtained from mice that were pre-treated with RES or vehicle followed by SEB exposure for 48hrs. The microRNA expression profile was determined to examine the epigenetic regulation and dysregulated genes were studied by scRNA-Seq analysis. scRNA-Seq analysis showed significant reduction in the expression as well as the number of cells expressing of mTOR and associated genes, RAC1, AKT, and Rictor, in various inflammatory cells in lungs of RES-treated when compared to vehicle-treated group. In contrast, Ppar-γ, Tgfβ-1 and Sirt2 gene expression as well as autophagy-related genes, ATG-13 and ULK1 were significantly higher in RES-treated group. miRNA array showed upregulation of miR-100 in T-cells treated with SEB+RES *in vivo* as well as *in vitro*. CD3+ T cells from lungs of T cell-specific AhR cKO or wild type mice treated with SEB+RES showed that miR-100-5p expression is AhR-dependent. Further, RES inhibited T-cell proliferation *in vitro* by blocking mTOR signaling and inducing β-oxidation in mitochondria instead of utilizing glucose as the main source of fuel which may be due to suppression of RAC1 mRNA controlling transport of glucose by glucose transporter 4 (GLUT4) vesicle. In addition, live-cell metabolic assay showed that bioenergetic profile utilized glucose-independent pathway and diminished glycolysis rate in RES-treated T-cells. We conclude that RES treatment ameliorated the ALI epigenetically either directly by metabolic reprogramming of activated T-cells by enhancing autophagy/apoptosis or indirectly by increasing anti-inflammatory cells. *Supported by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755, P20GM103641.*

1170 Role of Airway Club Cell Cytochrome P450-Mediated Bioactivation in Lung Epithelial Injury Induced by Naphthalene Inhalation

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Prior studies have clearly established that cytochrome P450 (CYP) enzymes in both lung and liver can bioactivate naphthalene (NA), a possible human carcinogen. In this study we examined the specific contribution of pulmonary CYPs in Club cells to the airway toxicity caused by inhaled NA. We used a lung-*Cpr*-null mouse model, which undergoes doxycycline-induced, Cre-mediated deletion of the *Cpr* (a redox partner of all microsomal CYPs) gene specifically in airway Club cells. In 2-month-old lung-*Cpr*-null mice, *Cpr* deletion occurred in 66% of Club cells in the proximal airways and 86% of Club cells in the distal airways, as indicated by the results of dual immunofluorescence staining of lung tissue sections for the Club cell marker CCSP (Club cell secretory protein) and CPR expression. Post-exposure toxicokinetic profiles of plasma NA and NA-GSH (a biomarker of NA bioactivation) were similar between lung-*Cpr*-null mice and their control littermates with normal CPR expression, following a single 4-h, nose-only, 10-ppm NA inhalation exposure. Total protein concentration and lactate dehydrogenase (LDH) activity in bronchioalveolar lavage fluid (BALF) collected 20-h after termination of active NA inhalation exposure were, respectively, 37% and 39% lower in lung-*Cpr*-null mice than in control littermates. The extent of incorporation of 5-bromo-2′-deoxyuridine (BrdU labeling index), which was significantly increased in both proximal and distal airways of all NA-exposed mice at 2 days post-exposure (by 4-13 fold, compared to filtered air-exposed, genotype-matched control groups), was 2-3 fold lower in lung-*Cpr*-null mice compared to control littermates. Similarly, genotype-related differences in sensitivity to NA-induced airway damage were also observed in mice after intraperitoneal administration of a single bolus dose of NA at 200 mg/kg. These results confirm that CYPs specifically localized to airway Club cells play a major role in NA-induced airway epithelial damage *in vivo* at an environmentally relevant NA concentration. *Supported in part by NIH grant ES020867.*

1171 Comparative Assessment of *In Vitro* Toxicity Induced by Crystalline Silica and Multiwalled Carbon Nanotubes in Human and Mouse Macrophages

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Pulmonary exposure to particles like crystalline silica (CS) and multi-walled carbon nanotubes (MWCNTs) are known occupational hazards. Once in the lung, these particles activate alveolar macrophages (AM), a first step in the complicated inflammatory cascade and development of diseases. To study the mechanisms involved in particle induced inflammation at the cellular level, human (THP-1) and mouse (RAW 264.7) macrophages were used as *in vitro* models and toxicity of CS and MWCNTs was tested. Cytotoxic dose-responses of each particle were determined at final concentrations of 0.9, 1.8, 3.7, 7.5, 15, 30, 60, 120, 240 and 480 μg/cm^2 for 24-hrs. Both particles caused a dose-dependent reduction in cell viability. Four concentrations pertaining to 0%, 10%, 30% and 60% toxicity for each cell and particle type were chosen for cytokine analysis: 41 markers in THP-1 and 32 markers in RAW 264.7 were measured. Activation of inflammasome cascade (IL18), pro-inflammatory markers (TNF-α), dysregulation (IL6) and fibrotic markers like growth factors, some of which play an important role in fibrosis, were observed. Cytokines involved in inflammation (IL1-B, IL18) and cell recruitment (MCP-1, MIP) were found to be elevated. Based on TH1/TH2 (IL18/IL4) ratio, there was a concentration-dependent polarization to TH1 response. Overall, THP-1 cells produced a greater inflammatory response to particle exposure than RAW 264.7 cells and MWCNTs were more potent than CS. Data clustering showed MWCNTs and CS treated THP-1 and RAW cells had 24 and 7 common cytokines, respectively, and 11 cytokines were found to be common between both cell lines and particle types. Principle component Analysis showed that the response, at same doses can be easily distinguished between particle type and was more apparent in THP-1 than RAW cells. In conclusion, both particle exposures resulted in significant cytotoxicity as well as production of inflammatory mediator in a concentration-dependent manner. However, THP -1 cells were more responsive than RAW cells and the potency of MWCNTs observed was much higher compared to silica at equal mass in both cell types, possibly due to difference in particle size and also due to difference in ASC inflammasome/casp1 activation cascade between two cell types. Ongoing transcriptomic studies may further differentiate the mechanisms of particle toxicity in different types of macrophages.

1172 *In Vitro* Toxicity Comparison of Surrogate Metal Oxides Found in Welding Fumes

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Welding fumes were classified as a Group 1 carcinogen (*carcinogenic to humans*) in 2017 by the International Agency for Research on Cancer based on sufficient epidemiological evidence and limited evidence in experimental animals. Toxic metals commonly found in the fumes are chromium (Cr), iron (Fe), and nickel (Ni). Copper (Cu)-based welding consumables are currently being investigated as a less toxic alternative. The objective of this study was to evaluate the acute toxicological potency of a new Cu-Ni welding fume in human bronchial epithelial cells (BEAS-2B) over a wide dose range (0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/ml). The primary components of the welding fume were also assayed to determine their relative potencies and included nickel (II) oxide (NiO; <10 μm and <50 nm sizes), copper (II) oxide (CuO; <10 μm and <50 nm sizes), and iron (III) oxide (Fe_2O_3; <5 μm). Physicochemical properties including dissolution were determined for the welding fume and its components. Membrane damage and cell proliferation/viability were quantified by measuring lactate dehydrogenase (LDH) release and conversion of the tetrazolium salt, WST-1, respectively after 24 h of exposure. The acellular oxidative potential of the welding fume and its component metals was determined by electron paramagnetic resonance. Cellular oxidative stress was measured via flow cytometry using change in CellROX. Experiments were run in a randomized complete block design (n=3 independent blocks) with a one-way layout of treatment combinations. The data showed that CuO (<50 nm) and Cu-Ni fume were the most toxic, and significantly increased LDH levels and decreased cell proliferation/viability with increasing concentrations *in vitro*. NiO (<50nm) was of intermediate toxicity primarily decreasing cell proliferation/viability at the higher doses with no significant effect on LDH levels. At equal mass, the nanocomponents were more toxic compared to their micron-sized components. No significant effects for damage and proliferation/viability were found for the other metal oxides tested. CuO (<10 μm and <50 nm sizes) and the Cu-Ni fume resulted in significant acellular and cellular levels of oxidative stress while the other metal oxides had no significant effect. These results suggest that the Cu-Ni fume has the potential to be inflammatory and toxic *in vivo*, and this effect may be primarily driven by the Cu component.

1173 *In Vitro* Toxicity Assessment of Respirable Solid Surface Composite Sawing Particles

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Solid surface composites (SSC) are a class of popular construction materials composed of aluminum trihydrate (ATH) and acrylic polymers. Previous investigations have demonstrated that sawing SSC releases substantial airborne dusts ranging size from 6 nm to 19.8 μm, with a geometric mean diameter of 1.05 μm. In mice, aspiration exposure to airborne SSC dusts induced symptoms of pulmonary inflammation at 24 h post-exposure: neutrophilic influx, alveolitis, and increased lactate dehydrogenase (LDH) and proinflammatory cytokine levels in lavage fluid. The particles appeared to be poorly-cleared, with 81% remaining at 14 days post-exposure. The objective of this study was to determine the toxicity of specifically respirable-sized particles on a model of human alveolar macrophages (THP-1). The relative toxicities of sub-fractions (0.07, 0.66, 1.58, 5.0, and 13.42 μm diameter) of the airborne particles were also determined. THP-1 macrophages were exposed for 24 h to respirable particles from sawing SSC (0, 12.5, 25, 50, or 100 μg/ml), or size-specific fractions (25, 50, and 100 μg/ml). Respirable particles decreased viability by 15% and 19% after exposure to 50 μg/ml and 100 μg/ml SSC, respectively, which correlated with increased cell culture supernatant LDH activity by 40% and 70% when compared to control. Reactive oxygen species (ROS) production were increased by 64% and 106% after exposure to 50 μg/ml and 100 μg/ml, and the glutathione peroxidase activity was increased by 22%, 18%, and 20% at the 12.5, 25, and 50 μg/mL exposure levels, respectively. IL-1β, IL-2, IL-4, IL-10, IL-12p70, IL-13, IFNγ, and TNFα were all increased in a dose-dependent manner. In the cells exposed to sub-fractions of SSC particles, at 50 μg/ml, the 0.07μm particles killed 23% of cells. At 100 μg/ml, 0.07 and 1.58 μm particles killed 36%, and 22% of cells, respectively. While each of these described fractions elicited a significant LDH response from control, they were not statistically different from each other. These results indicate a potential for cytotoxicity of respirable SSC particles and a relationship between particle size and toxicity.

1174 Identification of IL-17 Pathway as a Plausible Target for Pulmonary Effects following Acrolein Exposure Studies on Inbred Mouse Strains and Human Primary Bronchial Epithelial Cells at Air-Liquid Interface

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Acrolein is an important constituent of e.g. cigarette and biomass smoke. Chronic inhalation exposure has been associated with lung diseases like asthma and chronic obstructive pulmonary disease. Inter-strain variability in inflammatory response and oxidative stress was evaluated in seven inbred mice strains (129S1/SvImJ, A/J, BALB/cByJ, C3H/HeJ, C57BL/6J, DBA/2J, and FVB/NJ, females 12-14 weeks old) exposed to 0 or 1 ppm acrolein for 11 weeks (6 h/d, 5 d/wk). The *in vivo* experiments were followed up by acute exposure *in vitro* (0, 0.1 and 0.2 ppm acrolein, 30 min) using human primary bronchial epithelial cells (PBEC) cultured at air-liquid-interface (ALI). *In vivo*, total cell numbers in broncho-alveolar lavage and protein concentrations was unaffected by acrolein in all mouse strains. BALB/cByJ, C57BL/6J, and 129S1/SvImJ were the most affected strains with significantly increased expression of oxidative stress, pro-inflammatory and/or tissue injury markers. Both *Mmp9* and *Timp1* were significantly upregulated in the strains DBA/2J, C3H/HeJ and FVB/NJ indicating a change in protease-anti-protease balance. Upregulation of *Il17b* in the susceptible strains mice led us to investigate the pro-inflammatory IL-17 pathway genes in the PBEC-ALI model. *In vitro*, significantly increased expression of *IL17A, C and D*; *IL1β, IL22*, and *RORA* was detected in the PBEC-ALI following exposure to 0.1 and 0.2 ppm acrolein. The inter-strain differences in response to sub-chronic exposure to acrolein suggest that genetics may play a role in the pulmonary response to acrolein. Additionally, acrolein exposure mediated alteration of key IL-17 pathway genes in the PBEC-ALI model, which identifies IL-17 as a plausible candidate pathway warranting further mechanistic studies.

1175 Comparison of Vapor and Liquid Phase Acrolein Exposures to Air-Liquid Interface (ALI) Cell Cultures

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The 3M Strategic Toxicology Laboratory (STL) is an internal corporate resource that emphasizes the use of *in vitro* methodology when providing support to 3M businesses. The STL is investigating vapor and liquid phase exposure to cell cultures at the air-liquid interface (ALI) as an animal alternative assay to help assess respiratory toxicity. This study describes the use of acrolein, a potent respiratory toxicant, as a model test substance. Vapor atmospheres were generated using a Vitrocell® 12/12 system. A549 cells were seeded into Transwell® inserts, grown submerged for 1 week then raised to the ALI for an additional 7-14 days, creating cultures robust to the clean-air negative control conditions of the Vitrocell apparatus. EpiAirway™ tissues were purchased from MatTek. All exposures were carried out for three hours and viability assessed immediately or 20 - 24 hours post exposure. At the vapor phase, A549 cell viability was reduced to 58% (14.6 ppm) and 91% (4.6 ppm) compared with the Vitrocell clean-air control group, with a further decrease in each case of approximately 20% following the post-exposure period. Cell viability was not affected at the 1.46 ppm level. In the liquid phase, EpiAirway cultures were exposed to acrolein in corn oil ranging from 0.01 - 3,000 ug/mL. Viability decreased at all concentrations greater than 10 ug/mL. At 1,000 ug/mL, viability was reduced to 48% immediately and 4% 20 hours post exposure. A549 cultures were also exposed to acrolein dilutions in DPBS in the same range as EpiAirway and followed a similar viability profile. Both modes of exposure, vapor and liquid, demonstrated dose dependent effects on viability. However, the concentrations of acrolein required to decrease viability were much lower with vapor exposure than with direct liquid exposure. The liquid exposure level (1000 ug/ml) which produced approximately 50% reduction in viability (EC_{50}) converts to over 400,000 ppm in air, whereas 14.6 ppm vapor exposure reduced viability to approximately 58%. Further experiments are required to better understand the discrepancy in the liquid phase and vapor phase levels required to achieve the EC_{50} and to identify the most appropriate comparison method, such as concentration or mass/surface area. Understanding these differences will be critical to achieve the best utilization of these *in vitro* methods as a tool for respiratory toxicity assessment.

1176 Effect of Tyloxapol Treatment on % Active Cilia, Cilia Beating Frequency, and Mucociliary Clarance in MucilAir-Cystic Fibrosis Tissues

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The lungs are cleared of particles and kept sterile by cilia beating the mucus resulting in mucociliary clearance (MCC) of these materials. In cystic fibrosis (CF) patients, the mucus produced is thicker and, therefore, the MCC system does not function effectively. MucilAir™-Cystic Fibrosis tissues (MCF) are a functional model of the human airway epithelium derived from CF patients' cells cultured at the air-liquid interface resulting in a similar morphology to the patient, with functioning cilia, but defective MCC. The aim of the study was to measure the effects of the surfactant drug, Tyloxapol (TP), β-lactose (negative control) both in saline, and saline on the critical end-points for CF drug efficacy testing; % active cilia, cilia beating frequency (CBF) and MCC. Triplicate MCF were exposed to TP in saline at 0.0005 and 0.1% (v/v) over a 24 h time course alongside saline and β-lactose controls for 24 h only. Membrane integrity was measured pre and postdose by transepithelial electrical resistance (TEER). Displacement, a measure of MCC, was assessed by applying microbeads to the apical tissue surface, then movement tracked by video, with images analysed using TrackMate plugin on ImageJ software (NIH). CBF images were transferred to Epithelix CiliaX software to calculate % active cilia (>3.5 Hz) and CBF in all areas. Since % active cilia, CBF and MCC showed similar trends, only MCC, the final endpoint for measurement of drug treatments for CF, is discussed. All values are given as mean ± SD. The undosed displacement was 3.45 ± 7.04 µm. At 24 h, displacement increased for saline (52.9 ± 40.7 µm), β-lactose (36.2 ± 20.8 µm), low TP (62.1 ± 31.9 µm) and high TP (7.3 ± 7.4 µm). For the low dose timecourse, displacement increased to 20.8 ± 26.3 µm at 1 min and remained at this level until 1 h before increasing to 61.9 ± 34.0 µm at 2 h. For the high dose, displacement increased to 15.1 ± 11.5 µm at 1 min increasing to 35.0 ± 23.1 µm at 1 h before increasing to 68.3 ± 54.1 µm at 2 h. Toxicity in the high dose samples was observed as reduced TEER from 30 min post dose. Efficacy was partly due to the mucus thinning effect of saline used for all treatments, although the surfactant properties of TP increased this further. In conclusion, this study demonstrated that % active cilia, CBF and MCC can be measured and changes observed in M-CF. Therefore, it is proposed to use these endpoints in this test system to identify improved and novel treatments for MCC in CF.

1177 Gasoline Testing in VITROCELL 24/48 *In Vitro* Inhalation Exposure System as Alternative Method for *In Vivo* Inhalation Toxicity Studies

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There is an increasing demand to implement *in vitro* alternatives to *in vivo* experimentation in different research areas to comply with the 3R concept. As the inhalation route is one of the most relevant routes of exposure, alternatives approaches would need realistic lung cell models (*e.g.* 3D), realistic inhalation exposure systems (*i.e.* air-liquid interface (ALI)), and proper dosimetry techniques to increase the predictive ability of *in vitro* cell models and therefore accelerate the shift from *in vivo* towards *in vitro* testing. The ultimate goal of the 'PETRALI' project is to develop an alternative method for *in vivo* inhalation testing of petroleum-based substances. This work on gasoline testing was preceded by (i) development and validation of a generation facility to volatilize single compounds (*e.g.* ethylbenzene (EB)) and complex substances (*e.g.* gasoline); (ii) successful optimization and validation of VITROCELL® 24/48 exposure system for negative control (clean air ,CA), positive control (nitrogen dioxide, NO_2), and EB testing. Based on the pilot results using EB, an experimental set-up of 4 hour (h) gasoline aerosolization and deposition at ALI, followed by post-exposure incubation under ALI conditions (up to 24 h) has been established. The *in vitro* administered aerosol concentration of about 45.000 mg/m³ (converted LC50 rat for gasoline from *in vivo* to *in vitro*) could not be generated due to condensation of the gasoline vapour to 37°C needed for cell viability. So, A549 cells were exposed to a concentration-range of about 5000, 7500, and 10000 mg/m³ in 3 independent experimental runs. For each gasoline concentration two rows of 6 positions each were exposed. The flow per insert was 1.5 mlpm and trumpet height 2 mm. One row was exposed to CA and one row to NO_2 (about 20 ppm). Generation up to a maximum of 10000 mg/m³ gasoline gave no effect on A549 cell viability (MTT). Additional endpoints, such as inflammation and oxidative stress were measured. Exposure of A549 cells to 10000 mg/m³ gasoline induced an increase of pro-inflammatory markers *IL6* (log2 fold change (FC) = 1.94, p = 7.28E-3), *IL8* (log2 FC = 1.98, p = 2.18E-3), and *CCL2* (log2 FC = 1.13, p = 3.08E-3) as compared to CA, which was statistically significant for all markers. The oxidative stress marker *SOD2* was statistically significant increased for 10000 mg/m³ (log2 FC of ~ 0.56, borderline result). Administered dose could not be determined due to a combination of low deposition efficiency of the ALI system and detection limits off headspace GC-MS analysis for this complex mixture. Detailed results of the *in vitro* gasoline ALI exposure study and it's challenges/solutions for characterization will be highlighted. *The VITROCELL® 24/48* in vitro *inhalation exposure system was awarded to VITO by the PETA International Science Consortium.*

1178 Primary Human 3D Airway-Macrophage Culture Platform to Assess Toxicity of Suspended versus Aerosolized ZnO Nanoparticles

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The increasing use of nano-sized particles require an evaluation of their occupational exposure limit. Several studies demonstrated cytotoxicity and inflammation caused by metal nanoparticles, among them ZnO, which may increase the risk of pulmonary and also cardiovascular diseases. To enable a relevant *in vitro* prediction, complex primary human tissue models and realistic exposure methods are needed. We report an *in vitro* immunocompetent model based on air-liquid interface 3D fully differentiated human epithelia from upper (MucilAir™) or lower (SmallAir™) airway, co-cultured with human fibroblasts and surrogates of airway macrophages to evaluate inhalation toxicity of ZnO nanoparticles. The co-culture systems including basal, ciliated, goblet or club cells, fibroblasts and M0-like macrophages were successfully maintained and functional in a serum free culture medium (ImmunoAir™) for two weeks. The aim of the study was to compare the effects of aerosol and suspension exposure of ZnO nanoparticles on primary epithelial cells derived from human upper and lower airway with fibroblasts and macrophages using multi-endpoints analysis. Apical aerosol exposure of 1 µg/cm² ZnO (10-30 nm) was performed using a novel Vitrocell vial injector device using tetrafluoro-ethane as propellant, allowing uniform and real-time dose-controlled deposition. Dry exposure was compared to 1 and 10 µg/cm² apical suspension exposure in saline solution. Repeated 24 hours exposure were investigated during 3 days. Epithelial tissue integrity was well preserved in all conditions with a low cytotoxicity only on small airway model. Mucociliary dynamics was reduced and basal H_2O_2 production, Il-6 release were increased only on upper airway model. Dose dependent increase of Il-8 was observed

for both airway models. Amphiregulin, a growth factor implicated in epithelial tissue remodeling, was increased for both type of epithelia. Altogether, ZnO nanoparticles induces more robust changes via aerosol, than suspension exposure, and upper airway responds by multiple parameters, but without cytotoxicity, while small airway epithelia shows more cytotoxicity. These data suggest that this new generation of immunocompetent human airway models combined with aerosolized exposure may be useful for inhalation toxicity evaluation of airborne substances.

1179 Ciliopathic and Inflammatory Effect of Poly(I:C) on *In Vitro* 3D Human Airway Epithelium

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As first line of defense against airborne pathogens, the airway epithelium reacts through effective mucociliary clearance and secretion of inflammatory mediators. Poly (I:C), a mimetic of respiratory viruses, is capable of activating Toll-like receptor such as TLR3 on the airway epithelial cells. In this study, we would like to better understand the effect of Poly (I:C) on airway epithelial function using a multi-endpoint approach. An *in vitro* human airway model based on a pool of nasal cells from 14 donors (MucilAir™) was used to assess acute as well as repeated dose effect of Poly (I:C). The epithelial model is fully differentiated and tight, exhibiting efficient mucociliary clearance function (MCC). The tissues were exposed either to 9; 30 and 90 µg/cm^2 of Poly (I:C) apically during 96h, or to 30 µg/cm^2 of poly (I:C) every day during one week. Trans-epithelial electric resistance (TEER), cytotoxicity, and MCC were assessed simultaneously. Although no cytotoxicity was observed at all tested conditions, poly (I:C) induces a dose- dependent (i) decrease of TEER and (ii) reduction of MCC, correlated with a decrease of cilia beating frequency. The Inflammatory mediators such as Il-8, IL-6, IL-1β; RANTES and Interferon-λ are all reversibly upregulated in the context of single exposure, and also in case of one week repeated dose treatment. Altogether, Poly (I:C) induce a potent and typical inflammatory response with progressive loss of tissue integrity and mucociliary clearance function. Therefore, Poly (I:C) could be used as positive control to benchmark the effect of airborne substances on airway epithelium.

1180 Inter-Laboratory Ring Trial of the GARD Air Assay for Assessment of Respiratory Sensitizers—Results of Predictive Performance and Reproducibility

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Chemical sensitization is a disease state induced by the human immune system in response to chemical sensitizers, the proactive identification of which remains a central part of hazard and risk assessment of chemicals. Due to legislation, public opinion, concern for human environmental health and economic interests, there is an urgent need to develop animal-free methods for assessment of chemical sensitizers. While several assays for assessment of skin sensitizers have been proposed, the demand for an assay that accurately and specifically predicts and classifies chemical respiratory sensitizers remains unfulfilled. To this end, the novel assay GARD™air was developed, designed as an independent application of the GARD™ - Genomic Allergen Rapid Detection - technology platform. The GARD™ platform evaluates the transcriptional patterns of endpoint-specific genomic biomarker signatures in a human dendritic-like cell line following exposure, in order to provide machine learning-assisted classifications of tested substances. Specifically, the GARD™air prediction model is based on a set of genomic predictors related to the bridging of innate and adaptive immune functions and skewing towards Th2 type immune responses and has been previously been demonstrated to be functionally relevant, able to accurately classify respiratory sensitizers and differentiate them from skin sensitizers. Here, we present data from a recently performed inter-laboratory ring trial, aiming to estimate the within- and between-laboratory reproducibility (WLR and BLR), as well as the predictive performance (PP) of GARD™air. In summary, WLR ranged between 52-72%, while BLR was 79%. The PP was defined by an accumulated accuracy of 74% across the three different laboratories, with notable and consistent peak performances within subsets of the chemical space. Of further note, a specificity of 95% indicated that GARD™air is a potent screening tool for safety assessment of respiratory sensitization, providing a broad spectrum of industries with an affordable and more accurate alternative to current in-house (non-validated) animal-based methods.

1181 *In Vitro* Approach for Assessing Respiratory Toxicity in Human Lung Cells

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Approaches to efficiently and effectively assess the toxicity of chemicals on the human respiratory tract using *in vitro* systems would provide useful information to inform product development and risk management decisions. Presented here is an approach to help better understand the appropriate *in vitro* system to use and the biological markers to monitor based on the test chemical under evaluation. In this study, BEAS-2B cells (a human bronchial epithelial cell line) were exposed to various concentrations (0.72ppm, 25ppm, and 85ppm) of triethoxysilane vapor at the air-liquid interface using a capillary dosage unit coupled to a VITROCELL 6/4 exposure module. Triethoxysilane is an industrial chemical classified as a GHS category 2 inhalation toxicant based on rat acute inhalation toxicity testing. A significant concentration-dependent decrease in cell viability (resazurin-based assay) and increase in cytotoxicity (lactate dehydrogenase assay) was observed after exposure to the triethoxysilane (test chemical) and nitrogen dioxide (positive control) as compared to clean air (negative control). A significant increase in expression of inflammatory markers [interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α)], determined by Meso Scale Discovery technology (https://www.mesoscale.com/en), was observed at 25ppm. Additional work is underway to test other silanes that vary only in their carbon length to determine if this *in vitro* system can detect the decrease in toxicity that correlates with increasing carbon-chain length and to determine the advantages of using a 2D cell line (BEAS-2B cell) versus a 3D human reconstructed tissue model. Overall, these results will evaluate the utility of an *in vitro* system to predict the likelihood of a chemical to cause portal-of-entry effects on the human respiratory tract and could be a useful approach to rank chemical toxicity.

1182 Improving Respiratory Toxicity RD_{50} Prediction for Structurally Similar Chemicals

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The RD_{50} is the nominal air concentration leading to a 50% reduction in respiratory rate and has been used to establish airborne levels that are likely to provoke sensory irritation. Alarie (1981) defined the term sensory irritation to describe adverse health effects caused by compounds interacting with peripheral nerve fibers. Historically, the RD_{50} was experimentally determined *in vivo* where the chemical's potency as an acute respiratory irritant was measured through its ability to decrease respiratory frequency in laboratory mice following a short-term exposure. The *in silico* method for establishing a RD_{50} value for sensory irritation has remained largely unchanged over the past 25 years. As efforts to establish robust alternatives to animal testing for various endpoints progress, it is imperative that *in silico* predictions for respiratory toxicity continue to improve. In this present study, we build upon a previous calculation derived by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) using the octanol-water partition coefficient (K_{ow}) and the air-water partition coefficient (K_{aw}) to predict a RD_{50} for data poor chemicals. ECETOC observed that the accuracy of any given predicted RD_{50} using the generalized equation for all chemicals may be improved upon if statistical analyses were completed among a series of homologous chemicals. Thus, using a multiple linear regression approach, equations for predicting the RD_{50} for two structurally related groups of chemicals were generated. This group categorization enhanced the ability of K_{ow} and K_{aw} to predict the RD_{50} for aldehydes, ketones, and alcohols by 6.2% and for hydrocarbons by 2.1% when compared to ECETOC's generalized RD_{50} equation for all chemicals. This improvement of an *in silico* method for predicting sensory irritation will contribute to advances in alternative methods to animal testing and build upon our understanding of structure-related activity to fill data gaps for a predominately uncharacterized health endpoint.

1183 Inhalation of Acrolein Affects the Regulation of Mitochondrial Metabolism in the Airways

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Exposure of the airways to cigarette smoke is a known risk factor for developing several lung diseases including Chronic Obstructive Pulmonary Disease (COPD). Although cigarette smoke is a complex mixture of over 6000 chemicals, acrolein, a αβ1-unsaturated aldehyde generated during the pyrolysis and combustion of tobacco, is thought to be responsible for a large proportion of the non-cancer disease risk associated with smoking. Recently, cigarette smoke-induced mitochondrial dysfunction in airway epithelial cells has been implicated in the pathogenesis of COPD. While *in vitro* studies suggest that acrolein can impair mitochondrial function, whether or not inhalation of acrolein *in vivo* affects mitochondrial content or the pathways controlling this (mitochondrial biogenesis *vs* mitophagy) in cells of the airways is unknown. Therefore, in the present study, rats were exposed to acrolein inhalation (nose-only; 0-4 ppm), 4 hours/day for 1 or 2 consecutive days (n=6/group) and the activity and abundance of key constituents of mitochondrial metabolic pathways as well as expression of key proteins controlling mitochondrial biogenesis and mitophagy were investigated. Acrolein, dose- and time-dependently, affected the activity of enzymes involved in metabolic pathways, increased mtDNA copy number and decreased protein and transcript abundance of several subunits of complexes involved in the electron transport chain. Furthermore, protein and mRNA levels of key regulators of mitochondrial biogenesis were decreased in rat lung following exposure to the highest dose of acrolein. Protein and mRNA expression of components of the mitophagy machinery were unaltered in response to acrolein exposure. Collectively, these results demonstrate that acute acrolein exposure disrupts the molecular regulation of mitochondrial metabolism in rat lung. *Does not reflect the US EPA policy.*

1184 An Inexpensive Open-Source *In Vitro* Exposure System for Uniform Sedimentation of Liquid Aerosols Generated by New and Emerging Tobacco Products

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Multi-well *in vitro* aerosol exposure systems are necessary for investigating the toxicity of new and emerging tobacco products (NETPs). Most commercially available exposure systems rely on impaction, stagnation flow, or electrostatic manipulation of diluted aerosol to achieve particle deposition at cell culture surfaces. However, reported particle sizes of NETP emissions suggest these methods may not produce adequate deposition for toxicity testing. To address this, we developed and validated an inexpensive gravimetric sedimentation exposure system for puff-actuated NETPs. Our NETP Aerosol Settling Chamber, which can be housed within a standard cell culture incubator, uses microcontroller-based automation to deliver aerosols to a small (16cm x 9cm x 7cm) chamber containing a single multi-well plate. To quantify particle deposition, fiberglass filters were placed into 6.5mm Transwells and filter weights were recorded before and after a JUUL e-cigarette vape session (40, 5-second, puffs and 9-minute inter-puff settling time). A direct comparison was made with a Vitrocell VC10 stagnation flow system using the same experiment design and JUUL vape protocol. Sedimentation of JUUL aerosols in our system resulted in uniform particle deposition of 253µg ± 9µg onto each of 24, 6.5mm Transwells. The Vitrocell VC10 achieved 36µg ± 21µg deposition; significantly less than the deposition achieved by sedimentation and with greater inter-well variability. Based on these data, we believe our exposure system may provide an effective and inexpensive means for *in vitro* toxicity testing of NETP aerosols. Our intent is to publish a digital blueprint of this system, assembly instructions, parts list, and software code in an open-source format. Ultimately, we hope that this novel NETP Aerosol Settling Chamber will provide an improved and affordable approach for testing the toxicity of tobacco smoke, aerosols, and specific constituents in NETPs.

1185 A Proposed Framework to Assess the Respiratory Sensitization Potential of Isocyanate-Based Prepolymers

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The prediction of chemical respiratory sensitizers presents a challenge due to the lack of validated test guidelines and formally recognized *in vitro* or animal assays for this endpoint. Tiered weight of scientific evidence (WoE) approaches where skin sensitization data are evaluated as a first tier have been suggested and are routinely utilized. Using existing approaches, low molecular weight chemicals with a negative skin sensitization result are also considered negative for the potential to cause respiratory sensitization and further exploration of respiratory sensitization potential is considered only where positive skin sensitization results are observed or the substance is an isocyanate. Isocyanate-based polyurethane prepolymers, formed by combining an excess of diisocyanate (DII) monomer with polyol, exhibit properties (*i.e.*, higher molecular weight (MW) and viscosity, lower isocyanate content (%NCO) and vapor pressure) that decrease exposure relative to DII monomer. Local Lymph Node Assays (LLNA) conducted with low residual monomer toluene diisocyanate (TDI)-based prepolymers indicate that while lower MW prepolymers (950 and 1800 g/mole) exhibit evidence of sensitization potential (EC_3 0.11 and 7%, respectively), their potency is approximately 100-fold below that of TDI monomer (EC_3 ~0.02%) and a TDI-based prepolymer with a MW of 2950 g/mole was not interpreted to possess dermal sensitization potential based on comparison to mice treated with a similar concentration of residual TDI monomer. These results indicate that sensitizing activity is not solely related to %NCO and key physical and chemical parameters may influence sensitization potential. To evaluate the respiratory sensitization potential of isocyanate-based prepolymers, we propose a framework that is unique in its inclusion of further evaluation following a negative skin sensitization result in order to assess the potential for absorption in the lung. In our proposed framework, a negative skin sensitization result indicates that the prepolymer is neither a dermal sensitizer nor a respiratory sensitizer *via* the dermal route and triggers assessment of bioavailability, reactivity, biological/immunological WoE, and exposure to determine the potential risk. The proposed framework incorporates *in silico*, *in chemico* and *in vitro* methods and will facilitate an evaluation of the relationship between physical-chemical properties and respiratory sensitization potential.

1186 Toward the Replacement of Fetal Bovine Serum in Cell Culture Application: The Example of A549 Cells

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Foetal bovine serum (FBS), used as a supplement for cell culture media, presents significant scientific and animal-welfare concerns. The aim of this study is the complete replacement of animal derived components for cell culture application in the alveolar epithelial type II A549 cell line, which is commonly used in respiratory toxicology testing. A549 cells were transitioned to four different commercially available FBS free media: XerumFree™ XF212 (TNC BIO), HL-1™ (Lonza), X-VIVO™ 10 (Lonza), and CNT-PRA (CELLnTEC). After a successful transition into FBS free medium, different strategies were used to freeze cells (i.e. using 20% FBS + 10% DMSO, 10% DMSO only, DMSO + ProFreeze™ (Lonza), or CNT-CRYO-50 (CELLnTEC) freezing media). In parallel, TrypLE™ (Gibco) and TrypZean® (Lonza) were used as alternatives to trypsin to detached cells during cell maintenance. A549 cells did not survive the direct replacement of FBS; however, a gradual replacement (according to the suppliers' instructions) was successful for HL-1, X-VIVO 10 and CNT-PRA media. After several passages in FBS free conditions, A549 cells displayed different cell morphology depending on the media. Freezing cells in ProFreeze freezing medium showed results most similar to freezing the cells in FBS-containing media. The growth and functionality of the cells were compared between the alternative media and the control with 10% FBS. Following optimization of conditions, different strategies are possible for the replacement of animal derived components in the culture of A549 cells and that could be applied to many other cell lines. Use of chemically defined media represents a considerable advancement in cell culture that could overcome the reproducibility issues that result from different lots of FBS.

1187 A Model for Evaluating Synergistic Opioid-Induced Respiratory Depression

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Drug overdose is one of the leading cause of injury-related death in the United States with almost 68% of reported cases in 2017 involving a prescription or illicit opioid. Opioid overdose or combining opioids with other sedative medication causes respiratory depression leading to mortality. With the recent emergence of the opioid abuse crisis, it has become increasingly important to understand the potential interactive effects on respiratory parameters of new chemical entities (NCEs) that may be taken with opioids. For such assessments, positive and negative assay controls are useful to demonstrate proper evaluation. In this regard, respiratory function of male Sprague Dawley rats was assessed by plethysmography following administration of an intravenous (IV) morphine challenge (3 mg/kg) and/or oral baclofen (20 mg/kg). The conditions tested included oral vehicle (negative control), oral vehicle with IV saline (negative control), oral vehicle with IV morphine (positive control), oral baclofen alone (positive control), and oral baclofen concomitant with IV morphine (double positive control). In this model we demonstrate that administering morphine together with baclofen resulted in synergistic decreases in respiratory frequency (RF) and substantial decreases in minute volume (V) of 66% and 62% from baseline, compared to administering morphine (RF:17%; V:55%) or baclofen (RF: 38%; V: 48%) independently. Hence, baclofen with a morphine challenge model can be effectively used as a comparator to assess potential interactive effects of NCEs with opioids.

1188 Development of a Thermal Spray Coating Generator and Exposure System for Toxicology Studies

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Thermal spray coating is a surface treatment process that enables different types of materials to be deposited on various substrates- metal, metal alloys, ceramics, and plastics. The process involves spraying a metal coating product which is melted by high temperatures and then sprayed under pressure onto a surface. Applications for thermal spray processes have a broad range across all industrial sectors. This technique is widely used in repairing bridges, water towers, wind turbines, pipelines, and ship tankers as well as in the automobile and aircrafts industries. During the process, large quantities of aerosols composed of fine and ultrafine metal particles are generated. Little is known about the physical (e.g., particle size and morphology) and chemical (e.g., metal composition, solubility, surface chemistry, metallurgy) properties of the particles formed during the process. The goals of this research were to construct a thermal spray coating generator and exposure system for toxicology studies and to characterize the properties of the formed aerosols during different spray coating processes in a laboratory setting. Initial studies have evaluated twin-wire arc thermal spray coating using P-MET 730 stainless steel consumable wire. In twin-wire arc thermal spraying coating, two consumable metal wires are fed independently into the spray gun. The wires are charged, forming an arc between them. The heat from the arc melts the wires generating metal particles which are entrained in an air jet from a spray gun. The entrained molten metal is then deposited onto a rotary substrate via compressed air. Count median electric mobility diameter was measured to be 196 nm with a geometric standard deviation of 1.5, using a scanning mobility particle sizer (SMPS). Importantly, a significant portion of the primary particles were observed via scanning electron microscopy (SEM) to be in the ultrafine size range with diameters <100 nm. The particles were primarily composed of iron, chromium, nickel, and manganese. With the development of this system, it is possible to investigate the pulmonary and systemic health effects associated with the inhalation of aerosols generated from different thermal spray coating processes in an animal model.

1189 Effect of Polyhexamethylene Guanidine Phosphate on Epithelial Barrier Function in Human Bronchial Epithelial Cells

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Polyhexamethylene guanidine phosphate (PHMG-p) has been used as a disinfectant and biocide, and was reported to involve in lung diseases, such as pulmonary fibrosis. However, the effect of PHMG-p on the barrier function of the bronchial epithelium is unknown yet. Tight junctions (TJs), which comprise the interacting proteins, such as occludin, zona occludens-1 and claudins maintain epithelial barrier function. The damage of TJ is the major cause of epithelial barrier breakdown during lung inflammation. In this study, we investigated whether PHMG-p modulates the protein expression of TJ in human bronchial epithelial BEAS-2B cells. PHMG-p decreased the TJs and the other proteins including E-cadherin and β-catenin, which also maintain in barrier function in the epithelium. In addition, PHMG-p impaired the cellular F-actin architectures, involved in the maintenance of TJ structure and barrier function. Furthermore, PHMG-p increased the active calpain1, calcium-dependent protease, which is known to breakdown TJs. In addition, PHMG-p increased in the intracellular calcium levels via extracellular space. Interestingly, addition of calpain1 inhibitor, ALLN, and removal of calcium source in extracellular space significantly reversed the impairment of TJs and F-actin architectures induced by PHMG-p. These results suggest that epithelial barrier dysfunction is one of the symptoms to lead pulmonary diseases by PHMG-p via calpain1 activation.

1190 Early De-Risking Approaches for Identification of Anti-Fibrotics for Idiopathic Pulmonary Fibrosis (IPF) Treatment

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic progressing lung disease with a progressive and irreversible decline in lung function. It has a poor prognosis (3-5 yr survival from diagnosis) and increasing global prevalence (20 people per 100,000 population). To date, only Nintedanib (NINT) and Pirfenidone have been licensed to treat IPF, therefore there is increased effort to discover new therapies. NINT, a tyrosine kinase inhibitor was profiled in a fibroblast to myofibroblast human IPF lung assay to determine both efficacy and cytotoxicity using high content imaging. Myofibroblast formation is strongly associated with fibrotic lesions. NINT inhibited the generation of α smooth muscle actin formation (a marker of the transition to a myofibroblast phenotype) at doses of 30 nM and above, with an IC50 of 370 nM. Cytotoxicity (measured as changes in nuclei number, >50% reduction) was observed at >10 uM concentrations. In a bleomycin-induced lung fibrosis rat model, NINT dosed orally at 60 mg/kg BID inhibited lung fibrosis at lung concentrations of ca 100 nM (Median Modified Ashcroft Score reduced from 3 to 2, P<0.001, and hydroxyproline (collagen) levels in the lung were reduced by 30%, P<0.001). At these exposures mild side effects were observed, including reduced food consumption, lessened activity and body weight loss. These effects increased in severity with increasing dose and were seen in mouse as well as rat models. Hence, it may be possible to link early *in vitro* assessments of efficacy and cytotoxicity with *in vivo* pharmacokinetics and exposure estimates to help optimize the testing of compounds *in vivo* and de-risk compounds.

1191 The Role of Matrix Metalloproteinases in Multiwalled Carbon Nanotube (MWCNT)-Induced Inflammation in C57BL/6 Mice

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Matrix metalloproteinases (MMPs) are ubiquitously expressed extracellular matrix (ECM) proteases that are activated in inflammation and injury and play varied roles in cellular homeostasis, adaptation, tissue remodeling and immunity. We previously demonstrated that acute MWCNT exposure led to impaired vascular reactivity that was dependent on MMP-9 activation and CD36 signaling. We hypothesized that MWCNT inhalation exposure alters broad spectrum MMP activity, leading to serum-borne factors that enhance vascular permeability and activate inflammatory pathways. To test this hypothesis, male C57BL/6 mice (6-8 weeks) were given 10 mg/kg of the broad-spectrum MMP inhibitor, Marimastat, and then dosed with 0 (dispersion media; DM), 10 or 40 µg MWCNT via oropharyngeal aspiration 1 hour after Marimastat dosing. Pulmonary inflammation was evaluated 24 hrs following MWCNT exposure, in terms of cell and protein quantification of bronchoalveolar lavage fluid (BALF). Serum bioactivity was determined in mouse brain endothelial cells (MBEC) via serum cumulative inflammatory potential (SCIP) assay. Vascular integrity was evaluated via electric cell-substrate impedance sensing (ECIS) assay, and confocal assessment of intracellular gap formation. Neutrophil infiltration and total BALF protein significantly increased in 40 µg-dosed mice (n=6/group; p<0.01) and was not altered by MMP blockade. MBECs treated with serum from MWCNT-treated mice exhibited a ~75% reduction in barrier integrity compared to DM controls. A ~50% of DM recovery in barrier integrity was observed with MMP blockade. Additionally, MMP blockade diminished

MWCNT serum-enhanced thrombin-mediated loss of barrier integrity and enhanced sphingosine 1-phosphate-induced barrier stability in MBEC. Actin immunostaining of MBEC confirmed that MWCNT serum exposure disrupted cell-cell junctions in a MMP-dependent manner. A thrombospondin type-1 domain-containing peptide reduced regrowth of MBECs in a dose-dependent manner ($p<0.0001$). Thus, while MMP blockade did not alter lung inflammation resulting from MWCNT exposure, subsequent pathologic serum bioactivity arising from MWCNT treatment was dependent on pulmonary MMP activity.

1192 Use of Cell Media Nicotine Concentration as a Marker to Predict Cell Surface Deposited Nicotine in Transwells after Fresh Smoke/ Aerosol Exposure

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Exposure of organotypic 3D lung models at the air liquid interface (ALI) to fresh whole smoke/aerosol provides a more human relevant exposure assessment of combustible cigarettes and e-vapour products. The aim of this study is to present a method for determination of nicotine deposition at the 3D MucilAir™ tissue surface, in transwells of 24 MWP after repeated exposure to 30, 60 or 90 puffs of tobacco cigarette (3R4F, 1:17 dilution) or undiluted *my*blu™ e-vapour aerosol over 4 weeks. Exact measurement of nicotine deposited at the cell surface is difficult due to absorption of nicotine in to the cells. The nicotine serves as a general marker of exposure. We wanted to determine if cell media nicotine concentration was correlated with cell surface deposition. To measure this, the deposition efficiency onto a glass plate inserted directly into the transwell was determined and was compared to glass discs with cells grown on the surface (BEAS-2B, V79). During the four weeks of repeated exposure of MucilAir™ tissues at ALI to 3R4F smoke and *my*blu™ aerosol, basal culture media were collected for nicotine quantification. Additionally nicotine deposition on to glass discs representing the cell surface area was measured. Toxicological effects observed over the exposure time in comparison to the puff numbers were compared on nicotine basis. With regard to increasing puff numbers, dilution factors, and the surface area of glass plates nicotine deposition on glass discs in the transwells correlated well with the deposited nicotine in the cell media (measured using LC MS/MS). However the correlation coefficients obtained with the different products regarding nicotine deposition and nicotine concentration in the cell media deviated from each other due to the different physical characteristics of 3R4F smoke and myblu™ aerosol.

1193 DNA Damage from Regional Metal-Enriched Particulate Matter in a549 Lung Epithelial Cells

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Inhalation of particulate matter (PM) promotes the development of pulmonary diseases and cancer. Exposure to heavy metal-containing PM induces genotoxicity as mediated through the initiation of an inflammatory and oxidative response. However, the initial biochemical and physical interaction between PM and pulmonary epithelial cells that drives DNA damage is incompletely understood. This study therefore sought to develop an *in vitro* model to examine the genotoxic effect of PM on lung epithelial cells. PM was derived from 1) sediments from the Jackpile and St. Anthony uranium mines in NM; 2) from a church attic in Paguate, NM; and 3) tungsten carbide PM. All samples were resuspended in air and collected in a next-generation cascade impactor to ensure PM was respirable. Cells were labeled with a viability stain and a monoclonal antibody specific to the pH2AX histone variant, then imaged through the Cellinsight High-Content-Screening platform. H2AX fluorescence appeared greater in cells exposed to PM from the Jackpile mine, and dusts from the nearby church sample. Findings suggest that physical/chemical interaction with PM produce genotoxic effects on lung endothelium in the absence of inflammation. Further research is needed to elucidate underlying cellular mechanisms and putative physicochemical drivers of PM-induced genotoxicity.

1194 Effect of PHMG-p on Increasing of TRAIL-Related Death Receptors in Human Bronchial Epithelial Cells and Animal Model

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PHMG-p was used as the main component of the humidifier disinfectant to clean the humidifier and eliminate the growth of microorganisms. Epidemiological and toxicological studies have indicated that PHMG-p induces pulmonary inflammation and fibrosis caused by PHMG-p inhalation, but the toxicological mechanism of PHMG-p is still unknown. This study investigated whether PHMG-p-mediated upregulation of TNF-related apoptosis-inducing ligands (TRAIL) death receptors was associated with increased cell death. PHMG-p enhanced TRAIL-associated apoptosis, which correlated with upregulation of both TRAIL and TRAIL death receptor (DR) 4/5 expression through the induction of CHOP. PHMG-p enhanced the apoptosis-associated with extrinsic target protein (Fas and FADD) and intrinsic target protein (caspase-3, capase-8, and PARP). Also, PHMG-p promoted the pro-apoptotic (Bax) and reduced the anti-apoptotic (Bcl-2 and Bcl-xL) protein expression via upregulation of DNA damage associated proteins (H2AX and p53). Finally, PHMG-p increased Bid-activated cytochrome c release. Furthermore, the same symptoms as cells were seen in the PHMG-p-inhaled animal model. PHMG-p enhanced the apoptosis-associated with extrinsic and intrinsic protein target in mice. These results demonstrated the view that PHMG-p induces TRAIL-associated the cell death via the assembly of a death-induced signal complex (DISC) by TARIL-mediated DR4/5, FADD and caspase-8 activation in the early stages.

1195 The SILIFE Project: Production, Toxicity Screening, and Industrial Application of Quartz Species with Reduced Lung Toxicity

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In 1997 the IARC classified respirable crystalline silicas (RCS) as human carcinogens (category 1), strongly affecting work places in silica-dependent industries. IARC acknowledged differences among RCS species, based on source, chemical, thermal and mechanical history. As abundance, density and heterogeneity of surface silanol groups/radicals seem to be involved in RCS-mediated lung toxicity, adverse RCS lung effects might be reduced by coatings, covalently blocking these groups. Persistent problems with silicosis and the diversity of quartz applications stimulated issuance of the EU-project SILIFE, aiming at developing a dry surface-coating technology to abate RCS toxicity on industrial scale. The development process included choice of feasible, cost-effective coating additives (i.e. organosilanes and catalysts), definition of treatment parameters (technology, reaction time, dosage, application), proof of coating effectiveness and toxicity-reducing functionality by physico-chemical (e.g. ζ potential) and predictive *in vitro* and *in vivo* toxicity tests, with final implementation of coated RCS into industrial processes. Primary rat alveolar macrophages (4 h of incubation, 75 µg/cm^2 of pristine/coated quartzes) served as sensitive *in vitro* toxicity screening model with membrane (LDH-release) and DNA damage (Comet assay) as relevant endpoints. The very promising *in vitro* results were validated in a 28-/90-days intratracheal instillation study in male Wistar rats (pristine v. coated industrial quartz species; 2 mg/lung; positive control: quartz DQ12, 1 mg/lung). In bronchoalveolar lavage fluid both latency and variable, quartz species-dependent adverse reactivity of three industrial quartz samples was noted, using classical inflammatory parameters (differential cell count, LDH, ß-glucuronidase, total protein) and pro-inflammatory mediators (CINC-1, TXB_2) as meaningful readouts. But, more importantly, the study clearly demonstrated that some covalent RCS-coatings were indeed able to effectively block RCS lung toxicity in the rat for up to 90 days, without markedly compromising the technical process quality. Thus, covalent surface-coating of biologically reactive RCS species represents a promising strategy to render RCS handling safer. *Funding: LIFE 2014: project no. LIFE14 ENV/ES/00238.*

1196 Responses of Human Monocyte-Derived Macrophages and Differentiated THP-1 Cells Exposed to Silica Particles

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Macrophages, as professional phagocytes, represent a crucial cell component that surveys against injury and immune responses. *In vivo* they act as first line of defense and are involved in removal and clearance of (nano)particles but also their toxic effects e.g., by triggering reactive oxygen species production and inflammation. Here we evaluated the responses of human monocyte-derived macrophages (MDM) exposed to silica particles (DQ12, 2.1 µm) and compared with responses of the human cell line THP-1, which were differentiated into adherent macrophage-like cells (THP-1M) by phorbol-12-myristate-13-acetate (PMA). As endpoints we measured cellular ATP content, amounts of lactate dehydrogenase (LDH), tumor necrosis factor alpha (TNF-α), interleukin (IL-) -1α, IL-1β, and IL-8 in cell culture supernatants. MDM (12 donors) generated from blood monocytes by addition of granulocyte-macrophage colony-stimulating factor were exposed (24h) to increasing amounts of DQ12 (10-160 µg/cm²) and separately to lipopolysaccharide (LPS, from *E. coli* strain O55:B5) as positive control using exposure media with or without 10% fetal calf serum (FCS). ATP contents decreased (paralleled by increased LDH) with increasing particles amounts, namely at 40 µg/cm² to 50%, 25% after addition 80 µg/cm², and 2% in the presence of 160 µg/cm². Amounts of TNF-α reached 500 pg/mL TNF-α (range 2-15 fold of control) in both culture media after treatment with 80 µg/cm² DQ12. For comparison, LPS (0.3 to 3,000 EU/mL) increased TNF-α up to 20 ng/mL using medium with FCS. Increased amounts of IL-1α (up to 108 pg/mL, range 13-37 fold of control) were found after treatment with DQ12. In contrast, increased IL-1β amounts were only detected when cells were using FCS containing medium (up to 180 pg/mL, 2-9 fold), while IL-8 levels were not enhanced. THP-1M were exposed directly after their generation (without resting) and after 2 days of cultivation in PMA-free medium (with resting). DQ12 did not result in enhanced TNF-α amounts, while IL-1α was comparable with responses of MDM. Amounts of IL-1β in supernatants of THP-1M without resting were comparable to MDM, but were reduced after resting. Exposing THP-1M to high amounts of LPS found significant lower amounts of TNF-α compared to MDM, with THP-1M without resting being more comparable to MDM. Comparing responses to the model particle DQ12 and separately LPS, we conclude that THP-1M without resting may be a suitable model for studying macrophage functions and IL-1 α/ß responses. However, it is also necessary to point out that they have rather limited capacities to release TNF-α.

1197 Alternative Testing of Inhalable Aerosols: A Complementary *In Vitro/Ex Vivo* Model for Acute Inhalation Toxicity

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Sponsor: A. Bitsch

An *in vitro* model consisting of a human alveolar lung cell line (A549) in an air-lifted interface (ALI) culture situation and exposure using the P.R.I.T. ExpoCube device was combined in an experimental strategy with an *ex vivo* model consisting of an isolated perfused lung model (IPL) from the rat to achieve a testing strategy using only low amounts of testing materials (< 1 g), short testing periods and a high relevance of results as documented by correlation to literature data from acute inhalation toxicity *in vivo* animal testing. Test items for model validation included 5 commercial crop substances (Chlorothalonil, Captan, Mancozeb, Fosetyl-AL, Ethiprol) and two additional control items (sodium dodecyl sulfate (SDS) and n-dodecane). Test items were applied in a short-term / high concentration exposure design in both models. Acute inhalation toxicity was estimated from effects on cellular viability (*in vitro*) and on tidal volume and lung weight (*ex vivo*). Moreover, effects on the breathing mechanics could be explored using the *ex vivo* IPL approach. Dosimetry considerations were carried out on the basis of the specific particle deposition characteristics of the ExpoCube expose device (*in vitro*) respectively the multiple-path particle dosimetry model (MPPD, *ex vivo*) to estimate surface loads *in vitro* (µg/cm²) or lung loads (µg/lung) *ex vivo*. Comparison to *in vivo* literature data indicated highly correlated estimations of the acute toxicity *in vitro* in a nearly quantitative way without interference by test item solubilities also including the range of surface-load values *in vitro* and *in vivo* in the range of 1 to 100 µg/cm². *Ex vivo* results clearly enabled detection of harmful substances and, moreover, also enabled detection of test items exhibiting adverse effects on the mechanical breathing behavior, possibly by affecting the surfactant system of the lung (Fosetyl-AL, SDS, dodecane). In conclusion, it is proposed to apply the test system in a tiered approach (*in vitro*/*ex vivo*/*in vivo*) to achieve a relevant acute inhalation toxicity testing using only small amount of test substances and short testing periods in the sense of the "3R-principle" for replacing, refinement and reduction of animal experimentation.

1198 A Novel *In Vitro* Model to Assess Inhalation Toxicokinetics in a Multi-Organ Plate

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Aerosol generators have been used for years to assess respiratory toxicity of chemicals and nanoparticles *in vitro*. They allow for an *in vivo* like delivery of material to the lung tissue. Here we present the first characterization of an aerosol generator linked to a multi-organ platform (HuDMOP™) in order to model the systemic toxicokinetics of an inhaled material. The HuDMOP™ system is an integrated meso-scale, multiple organ *in vitro* system with simulated blood flow capable of modeling human systemic toxicity. Nicotine, was used for the characterization of this aerosol-to-multi-organ platform. First, wells containing only media were dosed with aerosolized nicotine either once (487 µg dose), or in a repeated dose format (three or six doses for 1460 µg and 2920 µg total dose, respectively) with each dose a minute apart. Media was sampled and analyzed by LC/MS to determine concentration of nicotine in the media and allow for percent (%) recovery calculations. The % recovery in the media was ~6.4% for all three exposure regimens. This was repeated with the aerosol generator closer to the media, which resulted in less recovery (~4.2%), suggesting the greater distance (4.3 cm) was optimal for maximal exposure. Finally, MucilAir™ tissues were linked to HepG2 cells in the HuDMOP™ system, creating a human lung-to-liver two organ system. The aerosol generator was connected and the MucilAir™ tissues were exposed to a single 487 µg dose of nicotine. The basolateral lung media, simulated blood, and liver media were sampled 1, 2, 3 and 6 hr after exposure, and analyzed by LC/MS. In addition, a blank (no lung tissue or liver cells) was run in order to assess the kinetics of nicotine without metabolism. In the presence of tissues/cells, nicotine peaked at 1 hr post-exposure (2157 ng/mL) in the basolateral lung compartment, then came to equilibrium at ~1100 ng/mL. In the liver compartment, nicotine increased slowly during the course of the 6 hr exposure, to a maximum of 63 ng/mL at 6 hr. In the simulated blood nicotine increased rapidly over the first 1 hr (169 ng/mL), after which the rate of increase slowed with a maximum accumulated concentration of 218 ng/mL at 3 hr. After 3 hr nicotine in the simulated blood decreased to 163 ng/mL at 6 hr. The kinetics of nicotine distribution were similar in the absence of cells, however, the overall levels of nicotine were higher, likely due to lack of metabolism. These results suggest that HuDMOP™ system linked to an aerosol generator can model systemic toxicokinetics of inhaled materials *in vitro*.

1199 Next Generation Risk Assessment Approach for Inhalation Exposures: Polymer Case Studies

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Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach that integrates new approach methodologies (NAMs) to assure human safety without animal data. We are currently evaluating a NGRA approach for inhalation exposures using hypothetical case studies of a film-forming polymer in personal care products (e.g. antiperspirants) and a silane in cleaning products. Impairment of mucociliary clearance, lung fibrosis and lung surfactant inhibition were identified as the relevant endpoints for the most common consumer exposure scenarios (e.g. daily use of an antiperspirant). To investigate these endpoints, two cell models were selected for *in vitro* testing: the MucilAir™-HF cell model (Epithelix) a system which shows ciliated as well as mucous producing cells typical for the bronchial region and the EpiAlveolar™ cell model (MatTek) a coculture system out of AT1 and AT2 (surfactant producing) cells, fibroblasts and THP1 cell representing the most common cells of the alveolar tract. In addition to the two case study chemicals another 16 benchmark substances were selected either due to their well-known effects in the specific areas of the lung, history of safe use and/or due to chemical or physical similarities to the case study chemicals. Linking the *in vitro* point of departures to the relevant *in vivo* consumer exposure level is essential to evaluate the usefulness of the *in vitro* test systems. Therefore, consumer habits and practises were used to derive an airborne concentration (mg/m³) for each chemical and exposure scenario, which was then transformed into deposited mass in the bronchial and alveolar region (µg/cm²) using MPPDv2.8. Cells were exposed daily for up to 12 days *in vitro* and different endpoints measured every other day. Preliminary results indi-

cate that the alveolar model was more sensitive to some of the pro-inflammatory benchmark substances tested. Polyhexamethylene guanidine phosphate for example induces a mild inflammatory response in the MucilAir™-HF system over the 12 days' treatment while inducing significant cytotoxicity in the EpiAlveolar™ cell model after only 4 days of exposure. Results on mucocilliary clearance are inconclusive since Benzalkonium chloride showed no significant effects.

1200 Prevention of Naphthalene Toxicity by Ergothioneine in Mice

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Naphthalene (NA), the simplest polycyclic aromatic hydrocarbon (PAH), is commonly found in mothballs, vehicle exhaust, wildfire and cigarette smoke. The effects of NA are site- and species-specific, with well-defined, dose-dependent Club cell toxicity in the mouse conducting airway epithelium, regardless of exposure route. Despite widespread exposure, few studies have examined efficacies of putative chemoprotective agents to limit the toxic effects of NA. Ergothioneine (ET), a thiol-thione found in mushrooms, has been reported to function as a cytoprotectant against oxidative stress in several systems. A specific ET transporter, known as the organic cation transporter novel type-1 (OCTN1) and encoded by the gene Slc22a4, has been identified as the main regulator for the uptake of cellular ET. This study aims to test the cytoprotective ability of dietary ET in the adult mouse lungs following NA exposure. C57BL/6J adult (2-3 months old) mice were exposed to an ET-free diet throughout the gestation and postnatal periods. They were then treated with an oral dosage of 70 mg/kg of ET, or saline, daily for 5 days. At 2-3 days following the ET treatment, the mice were given a single dose of NA (150 mg/kg) in corn oil, or corn oil alone, i.p. At 24 hours post NA injection, the lungs were processed for immunohistochemistry, high resolution histology, gene expression analysis, and metabolite analysis. The levels of ET were compared between saline (vehicle)- and ET-treated mice on the ET-free diet, to ascertain ET-treatment associated increases in circulating and lung tissue ET levels. Histology analysis at 24 hours post-NA treatment showed that ET-treated male mice were partially protected from NA-induced Club cell toxicity, compared to saline- and NA-treated mice. Levels of reduced glutathione (GSH) in the lung and liver at 24 hours post NA treatment were greater in ET-pretreated mice than in saline-pretreated mice, a result supporting the protective function of ET against NA-induced oxidant stress in the lungs. Additionally, gene expression of Slc22a4 in the lung was greater in ET-treated mice exposed to NA than in those exposed to corn oil. Furthermore, immunohistochemical analysis showed an apparent increase in OCTN1 immunoreactivity in the large airways and terminal bronchioles of NA-treated mice, compared to corn oil-treated mice, which may serve to facilitate cellular uptake of ET upon exposure to NA. *Supported in part by NIH grant ES020867 and T32 ES007059.*

1201 Physiochemical Characterization and Toxicological Assessment of Regional Particulates Adjacent Abandoned Uranium Mines within Native American Communities

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Particulate matter (PM) inhalation has adverse effects on the respiratory and cardiovascular systems. In sediments of abandoned uranium mines (AUMs), we found PM sized less than 2.5µm (small enough to enter the deep lung), in Northern Arizona (Claim 28) and Western New Mexico. Here, we determine the amount of the metal-rich PM in the ambient air at these locations, as well as the prevailing wind speed/direction with respect to surrounding communities, and immunological and physiological responses of mice after concentrated PM exposures. This will be the foundation to determine the relative toxicity of the ambient PM and its potential long-term health impacts. C57BL/6 and ApoE-deficient ($ApoE^{-/-}$; a vascular inflammation model) mice were exposed to concentrated ambient PM in a mobile laboratory located approximately 1 km southwest from the AUM, for 1 or 28 days for 4 hours/day to approximately 80 µg/m^3 PM. Lungs and aortas were collected for mRNA markers of inflammatory disease, followed by qPCR to gauge the possible inflammatory responses with inflammatory-specific probes. Bronchoalveolar lavage (BAL) was conducted to examine influx of macrophages and neutrophils into the lungs. Inflammatory response was also confirmed by mesoscale data analysis. No changes in BAL cellularity were noted and other indices of toxicity were modest. 1-day exposure qPCR showed increase in lung TNF-alpha and TGF-beta transcript levels in the PM group compared to filtered air (FA) mice. 28-day PM-exposed mice showed reduced CXCL1, TNF-alpha, and TGF-beta mRNA levels compared to FA mice. ICP-MS metals analysis of $PM_{2.5}$ in these exposures revealed a pattern of metallic species consistent with a crustal background (ie, no overt contamination from the mine sites). Wind stations placed above the mobile laboratory and mine sites showed a general wind direction from the southwest to northeast, which is generally away from the community near Claim 28. Currents effects seen are consistent with previous work of pulmonary inflammation related to PM exposure. Completion of exposures at contrasting mine sites give insight on the toxic potency of mine site-derived dusts and its prevalence in tribal communities.

1202 Accurate Identification of Estrogen Receptor in Mesothelioma Cell Lines Using High-Resolution Mass Spectrometry

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Asbestos is a carcinogen that causes mesothelioma a rare cancer that arises from the mesothelial lining of the pleura. Malignant pleural mesothelioma is an aggressive tumor that is resistant to conventional treatment including chemotherapy, surgery or radiation. Epidemiological observations show that for non-occupational exposure the incidence of mesothelioma is higher in women than men. However, women diagnosed with malignant pleural mesothelioma respond better to treatment and have a better prognosis than men. A recent study revealed that activation of estrogen receptor (ER)β decreased the growth of malignant mesothelioma (REN) cell xenografts. In contrary to this finding, our qPCR showed that three different malignant mesothelioma cell lines (MSTO-211H, REN, and IST) expressed ERα and ERβ at very low levels and this was supported by immunoblot analysis. However, the validity of ERα and ERβ antibodies has been an issue of concern. Herein, we developed an immunoprecipitation in gel-digestion liquid chromatography tandem mass spectrometric (IP-LC/MS/MS) proteomics method to detect ERα and ERβ in MCF-7 cells, and mesothelioma cell lysates. The presence of ER proteins was verified using accurate mass detection of tryptic peptides and MS/MS fragmentation patterns using a mass spectral library search in Skylines. Using this method, we successfully identified ERα and ERβ in HEK293 transfection cell lines. In MCF-7 cells we only detected ERα. However, there were no ERα or ERβ peptide fragments detected in mesothelioma cell lines. This finding indicated mesothelioma cell growth is not mediated by expression of ERα and ERβ. In the future, we aim to measure the expression of GPR30, a membrane-bound estrogen receptor protein, in malignant mesothelioma cells with our IP-based LC-MS/MS proteomics method. *Supported by P30-ES013508 from NIEHS.*

1203 Toxicological Effects of Soot Nanoparticles on the BALF of Alzheimer's Disease Model Mice

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Black carbon is a nanoscale anthropogenic air pollutant and is usually present in the form of soot that is formed in incomplete combustion processes. In addition to climate forcing effects, sooty aerosol is associated with exacerbation of several diseases, such as cardiovascular diseases, respiratory diseases, and neurodegenerative diseases like Alzheimer's disease (AD). The purpose of this study was to study adverse effects of nanoscale particulate matter in AD mice. In this study, we exposed 30 to 33 weeks old female transgenic (TG) 5xFAD mice and their wild-type (WT) female litter mates (n=12) to cast burner generated soot aerosol (GS) (32 nm mean diameter) or to clean air as a control. In this part of the study, we investigated the effects of particle exposure in the lungs, since the route of effects seen in the brain is unclear. With later analyses in the brain and other organs, including behavioral tests, we will have an overview of which exposure routes and biological mechanisms are the most important for brain health in aerosol exposure. From collected bronchoalveolar lavage fluid (BALF), total cell count, cell differential count, comet assay, and cytokine analysis (IFNγ, IL-10, IL-12p70, IL-1b, IL-2, IL-4, IL-5, IL-6, TNFα, and KC) were made. In addition to BALF, brains, intestine, and liver were collected and extensive analysis were made (not shown here). Genotoxicity in BALF cells shows slight variation between GS and clean air. In BALF cytokine concentrations (IFNγ, IL-10, IL-12p70, IL-1b, IL-2, IL-4, IL-5, IL-6, TNFα, and KC) some differences between exposure groups were seen. Engulfed soot agglomerates inside BALF macrophages were seen in GS group, which is an indicator for successful exposure.

1204 The Role of Long Non-Coding RNA, MALAT1, in Regulation of Oxidative Stress and Inflammation in a Mouse Model of Infant Respiratory Syncytial Virus Infection

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Human respiratory syncytial virus (hRSV) is a mucosal pathogen that affects virtually all infants by the age of 2 years and is responsible for 99% of childhood deaths that occur in developing countries. The global burden of hRSV in 2015 was estimated that 33.1 million children were infected, 3.2 million were admitted to hospitals, and 60,000 (<5 years) succumbed to illness. Currently, treatments for hRSV are limited and no vaccine is available, thus a greater understanding of hRSV pathogenesis is needed to target novel pathways to alleviate infection-associated sequelae. The long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has recently been suggested to act as a negative modulator of the Nrf2 transcription factor. MALAT1-defecient mice show significant upregulation of Nrf2-regulated antioxidant genes. Since the Nrf2 antioxidant response is critical to suppressing RSV pathogenesis, we sought to elucidate the role of MALAT1 in RSV infection using our neonatal mouse model that mimics infant infection. Five-day-old pups with *Malat1*$^{+/+}$, *Malat1*$^{+/-}$, or *Malat1*$^{-/-}$ backgrounds were infected with a chimeric RSV strain (rA2-19F). At 3 days post-infection pups were euthanized, and lungs were harvested in order to quantify pulmonary viral load. Pups were also sacrificed 9 days post-infection to characterize bronchoalveolar lavage fluid (BALF) cellularity and cytokine levels, lung and nasal epithelium histopathology, and pulmonary T cell profiles. Furthermore, there was no difference in total nucleated cell count collected from BALF, or body weights between groups. Work is ongoing to characterize the pathophysiological parameters post-infection.

1205 Endotoxin-Induced Mucous Cell Metaplasia in Mouse Pulmonary Airways Is Dependent on Innate Lymphoid Cells

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Inhalation exposure to gaseous and particulate environmental pollutants, including bacterial endotoxin (lipopolysaccharide; LPS), can cause an overproduction and hypersecretion of mucus that contributes to the severity of airway inflammatory diseases such as rhinitis and bronchiolitis. Airway responses to LPS include accumulation of neutrophils, mucin gene expression, and airway wall remodeling that ultimately results in a hypersecretory epithelium. We have recently discovered the requirement for innate lymphoid cells (ILCs) in ozone-induced mucous cell metaplasia in the nasal epithelium. In the present study we compared responses in lymphoid cell-sufficient C57BL/6 mice, ILC-sufficient Rag2-/- mice (devoid of T- and B-cells), and ILC-deficient Rag2-/-Il2rg-/- mice (devoid of all lymphoid cells) to test the hypothesis that the pathogenesis of LPS-induced mucous cell metaplasia (MCM) is dependent on ILCs. Mice were intranasally instilled with 0 or 10 µg of *E. coli*-derived LPS for 9 consecutive weekdays. Twenty-four hours after the last instillation, bronchoalveolar lavage fluid (BALF) was collected to determine inflammatory cell infiltration. Lungs tissues were collected gene expression analysis, and for morphometric determination of neutrophil density, and intraepithelial accumulations of mucosubstances and Club Cell Secretory Protein (CCSP). Airway exposure to LPS caused significant MCM in the lungs of ILC-sufficient mice (i.e., C57BL/6 and Rag2-/- mice) but not in ILC-deficient mice (Rag2-/-Il2rg-/- mice). In all mouse strains, LPS exposure was associated with a decrease in epithelial CCSP, and increases of neutrophils in BALF and airway tissues. Genes for mucin-associated proteins Muc5AC and ClCa3/GOB5, and for pro-inflammatory TNFα and IFNγ, were induced only in ILC-sufficient mice exposed to LPS. These results demonstrate that LPS-induced epithelial responses and mucus overproduction in mouse airways is dependent on ILCs. Further studies are needed to determine the subtype of innate lymphocytes (ILC-1, -2 or -3) that contributes to airway wall remodeling.

1206 Pharmacological CFTR Modulation Decreases Glycolytic Flux and Mitochondrial Respiration in Bronchial Epithelial Cells

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Cystic Fibrosis (CF) results from a mutation in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The CFTR is a chloride channel that is necessary for proper ion transport across epithelial surfaces, but also has indirect effects on a number of cellular processes including apoptosis, oxidative stress, and metabolism. CF patients suffer from a broad array of complications, including chronic bacterial infections and inflammation, leading to frequent pulmonary exacerbations and premature death. Additionally, individuals with CF possess dysfunctional mitochondria and display elevated markers of oxidative stress. Mitochondrial dysfunction has been identified across many pathologies and can often lead to apoptosis in high-energy cell types. The purpose of this study was to analyze energy metabolism in CF human bronchial epithelial cells (CFBE41o-; CFTR$^{\Delta F508}$) utilizing the Seahorse extracellular flux platform. This investigation revealed increased glycolytic flux and mitochondrial respiration, with significant increases in basal respiration, maximal respiration, proton leak, ATP production, non-glycolytic acidification rate, basal glycolysis, and glycolytic reserve in CFBE41o$^-$ cells compared to CFBE41o$^-$-corrected cells (CFTR^{WT}). To validate the role of mutant CFTR$^{\Delta F508}$ in the observed differences in energy metabolism, we exposed CFBE41o$^-$ cells to CFTR modulators Tezacaftor and Ivacaftor for 24 hrs. CFTR modulators are an important part of CF therapy and have provided significant improvement in lung function as measured by forced expiratory volume (FEV1). Combination Tezacaftor/Ivacaftor (Symdeko) was approved for use in CF in 2018 and is used to help correct the position of the CFTR in the apical membrane and improve chloride transport, respectively. Treatment of CFTR$^{\Delta F508}$ bronchial epithelial cells with the CFTR corrector or potentiator in mono- or combination treatment showed a general trend of reduced glycolytic flux and mitochondrial respiration. Additionally, we observed a significant reduction in ATP production and glycolytic reserve percentage with all treatments and significantly reduced basal mitochondrial respiration and coupling efficiency in cells treated with drugs in combination. Together these data show a synergistic effect of CFTR potentiation/modulation in lowering glycolytic flux and mitochondrial respiration in CF and the indirect role of the CFTR in energy metabolism.

1207 Real-Time Monitoring of the Adaptive Response to Oxidative Stress in Human Airway Epithelial Cells Exposed to an Environmental Peroxide

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Isoprene hydroxy hydroperoxide (ISOPOOH) is a major component of secondary organic aerosol formed by the photochemical oxidation of isoprene, the most abundant non-methane organic compound emitted into the troposphere. While previous studies have shown that ISOPOOH is a potent oxidant stressor of human airway epithelial cells (HAEC), the mechanisms involved are poorly understood. Intracellular redox homeostasis is supported by a high ratio of reduced to oxidized glutathione (GSH and GSSG, respectively) that is maintained at the expense of NADPH. In the present study, we used the genetically-encoded ratiometric sensor roGFP, which reports on the intracellular redox potential (E_{GSH} = RT/F log(2GSH/GSSH)) and iNAP1, which senses changes in NADPH, to monitor redox changes in HAEC undergoing controlled exposure to ISOPOOH in real time. Exposure of HAEC to ISOPOOH induced a pronounced oxidation of intracellular glutathione, that was temporally associated with a transient decrease in NADPH. The recovery of E_{GSH} and NADPH responses to baseline was initially complete but became impaired with repeated exposure to ISOPOOH, suggesting irreversible changes induced by sustained oxidative stress. Glucose deprivation of HAEC markedly potentiated E_{GSH} and NADPH responses induced by subsequent ISOPOOH exposures. Strikingly, the addition of 1 mM glucose following ISOPOOH challenge induced rapid and complete restoration of baseline NADPH and E_{GSH}. These findings reveal mechanisms involved in the cellular response to ISOPOOH and provide an unprecedented live view of the dynamic control of redox homeostasis in human lung cells exposed to environmental oxidative stress. *This abstract of a proposed presentation does not necessarily reflect US EPA policy.*

1208 Dynamic Expression of TRPV3 and Modulation of Wound Repair by Human Lung Bronchial Epithelial Cells

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Transient receptor potential vanilloid-3 (TRPV3) is a Ca^{2+} channel expressed by human lung epithelial cells. TRPV3 is activated by wood smoke particulate matter (WSPM) and it was proposed that TRPV3 may play roles in WSPM-induced cytotoxicity and damage repair based on the observation that mice treated sub-acutely with WSPM displayed bronchial hypersensitivity and

areas of epithelial hyperplasia, which were blocked by a TRPV3 antagonist. This study shows that TRPV3 expression is rapidly induced by HBEC3-KT and BEAS-2B human bronchial epithelial cells following monolayer disruption by trypsin-, scratch-, or WSPM treatment, further indicating a possible role for TRPV3 in coordinating early stages of the wound repair process. Using an *in vitro* scratch assay, it was found that TRPV3 antagonists "locked" cells into a non-migratory phenotype and prevented cell adhesion, presumably by blocking initiation of adhesion, migration, and other fundamental elements necessary for the wound repair process. Interestingly, over-expressing TRPV3 in BEAS-2B cells as well as TRPV3 agonists also attenuated wound repair. Transcriptomic profiling of BEAS-2B and BEAS-2B TRPV3-overexpressing cells revealed marked differences in the expression of epithelial-mesenchymal transition (EMT)-specific genes, and a downregulation of epidermal growth factor receptor (EGFR) receptors and growth factors. Supplementation of BEAS-2B TRPV3-overexpressing cells with the EGFR ligands HB-EGF and amphiregulin partially rescued this slow-repairing phenotype. Additionally, inhibition of EGFR and the kinase GSK3β attenuated TRPV3 expression following injury and slowed scratch repair. Finally, modulation of TRPV3 activity using agonists and antagonists influenced these and other EMT biomarkers including vimentin and E-cadherin. These results suggest a high degree of integration between TRPV3 and the canonical EGFR/EMT signaling networks during wound repair, which provide insights into how TRPV3 may influence lung epithelial repair after injury. These finding have both toxicological and therapeutic significance. *Support: ES017431 and ES027015.*

PS 1209 Assessment of Respiratory Health Symptoms and Asthma in Children Near a Drying Saline Lake

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Residents of the Imperial Valley, a rural, agricultural border region in California, have raised concerns over high rates of pediatric asthma symptoms. There is an urgent need to understand the influences and predictors of children's respiratory health in Imperial Valley. We assessed the impacts of sociodemographic, lifestyle, and household factors on children's respiratory health and asthma prevalence by administering a survey to parents of elementary school children (n = 357) in northern Imperial Valley. We observed an overall asthma prevalence of 22.4% and respiratory symptoms and allergies were widely reported, including wheezing (35.3%), allergies (36.1%), bronchitic symptoms (28.6%), and dry cough (33.3%). Asthmatics were significantly more likely to report respiratory symptoms, but high rates of wheezing, allergies, and dry cough were observed among non-asthmatics, suggesting the possibility for underdiagnosis of respiratory impairment in our school-age population. Having an asthmatic mother and exposure to environmental tobacco smoke were also associated with greater odds of asthma. Our findings provide evidence to support community concerns about children's respiratory health, while also suggesting that household and demographic characteristics have limited explanatory power for assessing asthma in this population. This work provides critical baseline data with which to evaluate local environmental factors and their influence on asthma and respiratory symptoms.

PS 1210 Immune Alteration in Neonatal Mice Born from Dams Intratracheally Exposed to Microplastics from Pregnant Day 9 to Neonatal Day 7

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Microplastics could be exposed through inhalation, oral intake, or skin absorption, which could lead to various health implications related with inflammation, genotoxicity, and oxidative stress. No systemic investigation has been conducted to evaluate immune toxicities involved with microplastics exposure, and furthermore, generation immune toxicity has not been reported yet. Immune modulation in neonatal day 8 ICR mice, which mice were born from dams intratracheally exposed to microplastics (polyethylene microspheres with 10~45 μm diameter) for 12 days from pregnant day 9 to neonatal day 7. Intratracheal instillation of the microplastics suspended in 3rd distilled water has been performed to three pregnant dams per group (60 or 6 μg/50 μl/instillation for high or low dose group, respectively), and control group was instilled with 3rd distilled water. Five neonatal mice per group were used for the evaluation. No significant difference in proportion of thymic $CD4^+CD8^+$, $CD4^+$, and $CD8^+$ T lymphocyte. Proportion of splenic helper T lymphocyte was lower in the low (27.6±3.0%) and high (32.3±4.1%) dose group than the control (35.6±4.1%), and cytotoxic T lymphocyte was also lowered in the low (14.5±1.7%) and high (13.9±1.8%) dose group than the control (15.9±1.5%), but B lymphocyte population was higher in the low (25.3±3.6%) and high (24.1±4.0%) dose group than the control (20.8±4.2%) without statistical significances. Considering the skewedness of helper T cell immune reactivity, splenocytes were stimulated with anti-CD3 mAb for 48 hours, and interferon-γ:IL-4 ratio in the culture supernatants was dose-dependently higher in the low (14.2±3.0) and high (16.9±4.6) dose group than the control (8.8±2.5). The level of TNF-α was also higher in the both low and high dose group than the control without statistical significance. The level of serum IgG1 was dose-dependently lowered in the low (0.38±0.02 mg/ml) and high (0.32±0.04 mg/ml) dose group than the control (0.47±0.08 mg/ml), which tendency was also observed with serum IgG2a level. The present study demonstrated the certain magnitude of immune alterations, especially inflammatory response in neonatal mice born from dams exposed to microplastics. *Supported by Ministry of Environment, the education program for the management of information on the hazard and risk of chemical substances.*

PS 1211 Glucocorticoids Rescue TGF-β1-Mediated β2-Adrenergic Receptor Dysfunction by Attenuating Gene Expression

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Glucocorticoids (GCs) and β-adrenergic receptor (β2AR) agonists decrease asthma symptoms in most patients. GC treatment results in gene expression changes in human airway smooth muscle (HASM), thereby modulating the inflammation and airway reactivity that are hallmarks of asthma. Our previous studies showed that the pro-fibrotic cytokine, transforming growth factor β-1 (TGF-β1), may be blunting the effects of intracellular cAMP to induce airway relaxation by increasing its breakdown through upregulation of enzymes called phosphodiesterases (PDEs). We hypothesize that dexamethasone (DEX), a GC, rescues TGF-β1-induced β2-agonist hyporesponsiveness by attenuating PDE4D gene expression in HASM cells. HASM cells were stimulated in serum-free F12 media with dexamethasone (DEX, 1000 nM; 30 min) prior to TGF-β1 (10 ng/mL; 18 hr). Subsequently, HASM cells were treated with β_2-agonist, isoproterenol (ISO, 1 μM; 5 min) or G_s activator, cholera toxin (CTX, 0.25 μg/mL). HASM cells were lysed and intracellular cAMP levels were determined by chemiluminescent immunoassay. RNA was isolated and purified from HASM cells using the RNeasy Mini Kit. cDNA was generated using SuperScript IV First-Strand Synthesis System. Relative cDNA quantification was performed using qRT-PCR. Overnight TGF-β1 treatment significantly decreased ISO-induced cAMP levels by 44.82% ± 8.56 (P=0.0064) and DEX rescued the reduction in cAMP levels induced by TGF-β1 treatment (25.83% ± 12.66; P=0.057 compared to TGF-β1 treatment). Additionally, TGF-β1 significantly attenuated CTX-induced cAMP levels (59.97% ± 10.01; P=0.004), which was then reversed by DEX pretreatment (71.47% ± 7.11; P=0.0017 compared to TGFβ1 treatment). Our RNAseq data shows little difference between *pde4d* expression in the presence or absence of DEX alone. We found that DEX (100 nM pretreatment) significantly decreases TGF-β1-induced *pde4d* expression (77.09% ± 8.17; P=0.0002). Our data show that TGF-β1 induces β2AR hyporesponsiveness through attenuation of β_2AR agonist- and G_s-induced cAMP production via *pde4d* upregulation in HASM. Reversal of the effects of TGF-β1 by DEX suggests a novel mechanism underlying GC-dependent effects on β2AR hyporesponsiveness in asthma.

PS 1212 Chronic Dermal Exposure to Soil Contaminated with Spent Engine Oil Induced Haematobiochemical and Spermatogenic Alterations in Wistar Rats

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Indiscriminate disposal of spent engine oil (SEO), a major source of environmental pollution in Nigeria poses physiological risk to humans and aquatic organisms. This study was aimed at investigating the effects of chronic exposure to SEO contaminated soil as beddings on the physiology of male Wistar rats. Animals were dermally exposed to soil contaminated with SEO (200, 300 and 400 mL) for 120 consecutive days and compared with uncontaminated soil (negative control). Heavy metal (Pb, Ni, Zn and Cd) accumulations, haematology, biochemical (AST, ALT, ALP, BUN and CREA) parameters, sperm morphology and histopathology (liver, kidney, lungs, brain, skin and testis) were evaluated. Results revealed that the heavy metals in SEO contaminated soil were far above the WHO permissible limits, with significant ($p < 0.05$) increases of Pb and Ni present in the brain, and Pb and Cd in the serum compared with the control. Significant ($p < 0.05$) increases in haematological (lym-

phocytes and neutrophils only) and biochemical (AST and ALP) parameters, and sperm abnormalities were observed in the SEO-contaminated soil compared with control. Histopathological changes were not evident in the brain but significant lesions were observed in the liver, kidney, skin and testis of exposed rats. Results herein suggest that the constituents of SEO contaminated soil could elicit harmful physiological effects to humans who are intentionally or mistakenly exposed to spent engine oil.

1213 Potentiation of Dioxin-Induced Edema by LPS in Larval Zebrafish

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It is not well understood that inflammatory responses triggered by Toll like receptor (TLR) agonists in eleutheroembryos of teleost including zebrafish that lack adaptive immune system. Otherwise, involvement of aryl hydrocarbon receptor (AhR) has been reported in inflammatory responses by TLR agonists in mammalian system. In this study, the effects of lipopolysaccharide (LPS), one of representative TLR agonists on early edema caused by TCDD in larval zebrafish were investigated. Water-borne exposed LPS did not affect edema by itself but augmented edema caused by lower concentration of TCDD in a concentration-dependent manner. LPS even caused edema in the presence of very low concentration of TCDD that never induced edema. Edema caused by LPS plus very low concentration of TCDD (LPS+low TCDD) were abolished by cyclooxygenase 2 (COX2) inhibitor, a thromboxane receptor (TP) antagonist and prostacyclin receptor agonist, all of which inhibited high concentration of TCDD-induced edema. Edema caused by LPS+low TCDD were abolished by an antioxidant and activator of Nrf2, a master regulator of anti-oxidative responses. LPS also enhanced edema caused by TP agonist in antioxidant- and Nrf2 inducer-sensitive manner. LPS did not affect expression of type 2 AhR, CYP1A, markers for neutrophils and macrophages, and interleukin-6 and TNFα irrespective of co-treatment of TCDD. Reduction of neutrophils and macrophages by morpholino antisense oligos to G-CSF receptor and PU.1/Spi1 did not affect edema caused by LPS+TCDD. These results suggest that LPS augments TP receptor signaling through oxidative stress resulting in potentiation of edema by TCDD in developing zebrafish.

1214 Non-Monotonic Metabolic Dysregulation by Chronic Exposure of Low-Level Organochlorine Pesticides Mixture

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Exposure of organochlorine pesticides (OCPs), a class of persistent organic pollutants, has shown strong relationship with metabolic syndrome such as type 2 diabetes and obesity in epidemiological studies. To demonstrate metabolic adverse effects of OCPs mixture, 12 months-old female and male zebrafish adults were exposed to 0, 0.05, 0.25, 2.5, and 25 μg/L of five predominant organochlorine (i.e., p,p'-DDT, chlordane, heptachlor, beta-HCH (beta-hexachlorocyclohexane), and hexachlorobenzene (HCB)), for three months using an automatic flow-through exposure system. We measured typical blood index for metabolic effects (i.e., glucose, insulin, triglyceride, and free fatty acid level), and analyzed the alteration of gene expression through RNA-sequencing. The significant change of blood sugar regulation (i.e., high glucose and low insulin level) was observed non-monotonically at lower concentration of 0.05 μg/L than 0.5 and 25 μg/L, in only female zebrafish. These phenotypic changes were closely consistent with the transcripts differentially expressed by exposure of OCPs mixture. The metabolic pathways on PPAR-signaling, mitophagy, insulin signaling, lipid metabolism, glycolysis and carbohydrate metabolic process (i.e., citrate cycle, pyruvate metabolism) were found to be significantly affected in the transcription level. Therefore, our results suggest that chronic exposure of low-level OCPs leads to metabolic dysregulation, especially diabetic effects, in a non-monotonic manner in female zebrafish.

1215 Early-Life PBDE Exposure in Mice Persistently Dysregulates Intermediary Metabolites and Expression of Xenobiotic Biotransformation and Epigenetic Genes in Adult Liver

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Exposures to environmental toxicants during development can alter adult disease risks. The former flame retardants polybrominated diphenyl ethers (PBDEs) are bio-accumulative and readily detectable in humans. We have previously shown that acute exposure to PBDEs in adult mice alters abundances of hepatic intermediary metabolites and expression of xenobiotic biotransformation genes. Here, we tested our hypothesis that short-term exposures to PBDEs in neonatal mice persistently dysregulates hepatic intermediary metabolism and expression of xenobiotic biotransformation genes into adulthood. During postnatal days (PND) 2-3, male and female C57BL/6J mice were exposed to PBDE congeners BDE-47 (5, 50, 150 μmol/kg), BDE-99 (1, 5, 50 μmol/kg), or corn oil (vehicle, 10 ul/g) once per day via intraperitoneal injection. Tissues were collected at PND5, PND15 and 30 (adolescent ages), and PND60 (adulthood). Liver gene expression was quantified with RNA-Seq. Reads were aligned to mm10 using HISAT2. Genes were counted using featureCounts. DESEQ2 was used for differential expression analysis. LC-MS was used to measure hepatic aqueous metabolites. We found that early-life exposure to BDE-47 and -99 increased the abundances of amino acid, nucleotide, and carbohydrate metabolites in the livers of adult male and female mice at PND60. Expression of xenobiotic biotransformation genes including Cyp2, Cyp4, Gst, and Ugt isoforms and intermediary metabolism genes including Hk2 was down-regulated. Overall, BDE-99 altered the abundances of more hepatic metabolites and transcripts than BDE-47. However, the magnitude and direction varied by dose and sex. Notably, low-dose BDE-99 males exhibited the largest number of dysregulated metabolites and genes. In contrast both congeners at all doses down-regulated genes in females at PND60. Genes that epigenetically regulate transcription, including histone lysine demethylases (Kdm) and Pdp1 which coordinates certain methyltransferases, were also dysregulated through Day60. Significant correlations were observed between epigenetic genes Kdm5d and Pdp1 and P450 isoforms (Cyp2a and Cyp4a) and Hk2 both at PND60. In summary, early-life PBDE exposure persistently altered adult mouse intermediary metabolism and xenobiotic gene expression in a congener-, sex-, dose-dependent manner. Correlations between PBDE-regulated epigenetic genes and xenobiotic biotransformation and intermediary metabolism genes suggest that epigenetic modifications likely contribute to the sustained nature of observed PBDE effects on metabolite abundances and gene expression.

1216 Effect of Polychlorinated Biphenyl (PCB) Exposure on Mitochondrial DNA (mtDNA) Copy Number

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Mitochondria are intracellular organelles that contain their own genome and are responsible for biological oxidation and energy production. Mitochondrial DNA (mtDNA) is highly susceptible to damage from oxidative stress and exposure to polychlorinated biphenyls (PCBs) has been shown to significantly increase oxidative stress. The copy number of mtDNA varies depending on cell type and can be modulated by changes in physiological conditions or mitochondrial damage. The National Toxicology Program (NTP) conducted two year studies exposing female Sprague Dawley rats to various doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the dioxin-like compounds (DLC) PCB 126 and PCB 118, the non-DLC PCB 153, and a mixture of PCB 126 and PCB 153. mtDNA copy number was assessed by quantitative PCR utilizing DNA that was isolated from banked liver and lung tissue obtained from the NTP studies. Relative to time-matched vehicle controls, the changes in mtDNA copy number observed varied depending on the exposure compound, exposure length, dose, and tissue type. Following 13 weeks of TCDD exposure there were significant increases in mtDNA copy number in the liver of animals exposed at the two highest doses. Similarly, 30 weeks of TCDD exposure at the two highest doses resulted in significant increases in mtDNA copy number in lung tissue. This significant increase was also seen in the lung tissues of animals exposed to the highest dose of TCDD for 52 weeks. Similarly, exposure to PCB 118 caused significant increases in mtDNA copy number in liver and lung tissues of animals exposed at the two highest doses for 13 weeks. Exposure to PCB 126 at the lowest dose tested showed significant decreases in mtDNA copy number in lung tissues of animals exposed for 13 and 52 weeks. Following an exposure to PCB 153 there was a trend toward decreases in mtDNA copy number. 52 weeks of exposure to the mixture of PCB 126 and

PCB 153 showed a significant increase in mtDNA copy number in liver of animals exposed to a mid-level dose, while the highest dose caused a significant decrease in mtDNA copy number. Changes in mtDNA copy number can serve as a biomarker that may be associated with neoplastic and/or non-neoplastic responses observed with subchronic and chronic exposures to TCDD and PCBs. *Supported in part by the intramural research program of NCI/NIH.*

1217 Keyword Analysis Tool: A Case Study Analysis of Congener-Specific Half-Lives for Polychlorinated Biphenyls (PCBs)

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Toxicity resulting from human exposure to PCBs is highly dependent on the absorption, distribution, metabolism, and excretion (ADME) properties of the individual PCB congeners. A database of half-life of elimination values for individual PCB congeners was assembled to advance the development of a comprehensive kinetic model for PCB mixtures relevant to human exposures. A novel strategy was employed to identify relevant literature. First, a literature search of the PubMed, Web of Science, and ToxLine databases was conducted using keywords related to PCBs; this generated a database of 58,051 studies. Next, the Keyword Analysis Tool (KAT) was applied to select studies related to PCB half-life and elimination. KAT is a software tool designed to increase efficiency in literature screening; it allows users to calculate metrics on the utility of each keyword. By applying keywords related to half-life, elimination, and clearance, KAT identified 5,956 potentially relevant studies from the PCB literature database. A text analytics approach was implemented to further select for studies considered relevant for data extraction based on an evaluation of PCB half-life or elimination rates in humans or other mammals. Specifically, sentences from the full text of each study that harbored a keyword were reviewed in conjunction with titles and abstracts to ascertain study relevance. This effort identified 312 potentially relevant studies, 98 of which contained half-life values for 95 PCB congeners across all species, lifestages, biological matrices, and exposure routes. A retrospective analysis of the KAT search results was undertaken to measure the utility of each keyword alone and in combinations in retrieving relevant studies. Of the 64 keywords used, only 28 were associated with studies considered relevant, and the top 10 keywords identified almost 80% of studies with half-life or elimination data. Additionally, by including keywords relevant to ecological studies, KAT successfully identified studies of PCB elimination in non-biological matrices such as air, water, and soil, which helped prioritize other studies more likely to be relevant to PCB elimination in mammalian species. Thus, KAT identified a highly effective keyword set that could be applied to increase efficiency of future literature searches for chemical half-lives of elimination in humans and other mammals.

1218 A Broadly Applicable Method for Identification and Analysis of Polychlorinated Biphenyl Sulfates in Human Serum

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Polychlorinated biphenyls (PCBs) are a class of toxic persistent chemicals with both legacy sources (e.g., Aroclors) and new current sources (e.g., unintentionally produced contaminants in some pigments and varnishes). PCB sulfates are derived from further metabolism of hydroxylated PCBs (OH-PCBs), which are oxidative metabolites of PCBs. Both OH-PCBs and PCB sulfates exert multiple toxicological effects on human health such as disrupting thyroid hormone transport and inhibiting steroid sulfotransferases (SULT1E1 and SULT2A1). Although PCB 11 sulfate has been previously detected in human serum samples by LC-MS, this is not a generally applicable method for a broad range of PCB sulfates in human serum (e.g, the theoretical number of mono-hydroxylated PCBs from which these PCB sulfates might arise is 837 congeners). This has limited our understanding of their prevalence and importance. We propose a method that employs acetonitrile extraction of the PCB sulfates from serum followed by differential analysis with, and without, hydrolysis to OH-PCBs catalyzed by a purified sulfatase from *Helix pomatia*. After purification of the sulfatase by affinity chromatography, its broad specificity for PCB sulfates indicated the feasibility of its use for their quantitative hydrolysis to OH-PCBs. These OH-PCBs were quantitated by GC/MS/MS after derivatization to their corresponding methoxy PCBs. Using 13C-OH-PCB 29 as surrogate standard, we were able to recover 64 ± 4 % of 10 ng of 4-PCB 11 sulfate added to 2 g of pooled human serum. An initial evaluation of this method led to the observation of several PCB sulfates, including 4′-PCB 26 sulfate, 3-PCB 54 sulfate, 4′-PCB 25 sulfate, and 4-PCB 52 sulfate, in a pooled human serum sample. Further analyses of serum samples for PCB sulfates are in progress. *Supported by NIH: P42 ES013661.*

1219 Analyzing the Effect of Pooled Samples on Interpreting the NHANES Dioxin Serum Data

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From 1999–2004, blood samples from individuals in the National Health and Nutrition Examination Survey (NHANES) were collected and analyzed as individual measurements. Beginning in the 2005-2006 cycle, blood samples from eight individuals were pooled into a single sample based on demographic characteristics and analyzed as a whole. This resulted in a significant reduction in limits of detection (LODs), which could make it difficult to compare the NHANES congener and toxic equivalency (TEQ) data from individual (1999–2004) to pooled (2005–6) biennials. Specifically, it has been suggested that there may be artificial decreases in mean congener and/or TEQ concentrations in the pooled data due to the lower LODs; this concern would be particularly applicable to congeners with a low detection frequency (DF) in the individual biennials but a high DF in the pooled biennials (non-detect values are approximated by substituting the LOD/square root of 2). The purpose of this analysis was to assess the influence that sample pooling may have had on NHANES data interpretation. It was found that the LODs of all congeners decreased substantially (up to 154-fold) as a result of the transition from individual to pooled samples (2003/4 vs 2005/6). However, it was also found that in white 60+ males, there was no difference in the congener fingerprint (% contribution to total mass) of the individual vs. pooled data. Specifically, the congeners OCDD, 123678HxCDD, and 1234678HpCDD comprised approximately 71%, 9% and 9% of the total congener mass (respectively) in both biennials. Time trend analyses also show that the blood levels of the congeners that comprise the majority of the TEQ in any given biennial (12378PeCDD, 123678HxCDD, 23478PeCDF, and 2378TCDD) did not exhibit an implausible decrease in the transition from individual to pooled data; indeed it would appear that blood concentrations of 12378PeCDD, 23478PeCDF, and 2378TCDD have remained stable in white 60+ males since the 2003/4 individual biennial. These congeners have relatively high DF in both the individual and pooled data, and as a result the transition to significantly lower LODs had little impact on the blood TEQ. The congeners associated with the largest decreases in mean values do not contribute substantially to the TEQ. These findings suggest that it may be appropriate to include all NHANES sampling cycles (individual and pooled) when investigating time trends of blood TEQ or the individual congeners that comprise the majority of TEQ.

1220 Evaluating the Effects of the Persistent Organic Pollutant Aroclor 1260 on Gut Microbiome in CAR and PXR Knockout Mice

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Polychlorinated biphenyls (PCBs) are persistent organic pollutants associated with multiple health problems in exposed population, including nonalcoholic fatty liver disease (NAFLD). Previously, our group demonstrated that exposure to the PCB mixture, Aroclor 1260 (Ar), exacerbated NAFLD in diet-induced obese mice and activated the pregnane-xenobiotic receptor (PXR) and constitutive androstane receptor (CAR). Recent studies have also reported that PCBs can induce changes in gut microbiome, thereby promoting liver toxicity and worsening NAFLD. The objective of this study is to examine PCB effects on the gut-liver axis and characterize the role of CAR and PXR in PCB-induced alterations on microbiome and intestinal function. Methods: C57Bl/6 (wild-type, WT), $PXR^{-/-}$ and $CAR^{-/-}$ mice were fed a high fat diet (HFD) for 12 weeks and exposed to corn oil or Ar (20 mg/kg) *via* oral gavage. Cecal and ileal samples were collected for 16S rDNA sequencing and RT-PCR analysis. Results**:** Metagenomics analysis revealed that the bacterial species (alpha diversity) in WT mice were significantly different from both $PXR^{-/-}$ and $CAR^{-/-}$ mice; implicating involvement of these receptors in bacterial homeostasis. $PXR^{-/-}$ and $CAR^{-/-}$ mice also exhibited higher beta diversity than WT mice, irrespective of Ar exposure. Additionally, Ar induced changes in bacterial richness and diversity between groups in WT, $PXR^{-/-}$ and $CAR^{-/-}$ mice. In terms of bacterial composition, both knockout groups displayed decreased firmicutes/bacteroidetes ratio, accompanied by increased relative abundance for bacteroidetes, verrucomicrobia and TM7, and short chain fatty acid producers including bifidobacterium and lacto*Bacillus*. Further analysis of ileal gene expression showed that Ar decreased tight junction protein (*Tjp1*) mRNA levels in the knockout *vs.* WT groups. Moreover, $CAR^{-/-}$ mice exposed to Ar had decreased mRNA levels for genes encoding intestinal permeability and function markers including occludin, claudin3 and mucin2. Conclusion: Taken together, the results suggested that CAR and PXR ablation as well as Ar exposure modified

gut bacterial diversity; Ar also appeared to compromise intestinal integrity/function with CAR ablation. Future studies include further analysis of bacterial composition related to inflammation, lipid and bile acid metabolism to gain more insight into the observed gut dysbiosis.

PS 1221 The Ionic Liquid M8OI (C8[mim]) Inhibits Complex I of the Mitochondrial Electron Transport Chain

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Ionic liquids are organic salts that have been proposed as a "green" alternative for currently used solvents due to their low volatility and other desirable physicochemical properties. However, they may persist in the environment due to their water solubility and low biodegradability. Recent environmental sampling around a landfill site in the north east of England discovered high levels of the ionic liquid 1-octyl-3-methylimidazolium (M8OI, C8[mim]) based on its inhibition of oxygen consumption and toxicity to a liver progenitor cell. It was proposed that this chemical could adversely affect the liver and trigger cholestatic-related disease such as primary biliary cholangitis (PBC). It was therefore hypothesised that M8OI interacted with a component of the mitochondrial electron transport chain, inhibiting oxidative phosphorylation, increasing ROS production to induce apoptosis. To test this hypothesis, the rat hepatic progenitor B-13 cell line was exposed to M8OI. Exposure after 24 hours reduced cell viability (EC50 = 10μM) and induced apoptosis (≥100μM). Complex specific activity was measured in permeabilised cells using extracellular flux analysis. M8OI inhibited oxygen consumption in the presence of a complex I-linked substrate. Oxygen consumption in the presence of the complex-II linked substrate, succinate, was not inhibited by M8OI, suggesting complexes II, III and IV are not involved in the mechanism of inhibiting oxidative phosphorylation. Measurement of oxygen consumption through complex III (using duroquinol as an electron donor) and complex IV (using tetramethyl-*p*-phenylene diamine as an electron donor to cytochrome c) confirmed M8OI did not interact with either of these complexes. To confirm the inhibition of complex I by M8OI, hydrogen peroxide release from isolated mitochondria was measured using the fluorescent probe amplex red. In the presence of complex I substrate, M8OI increased H_2O_2 release, and in the presence complex II-linked substrate, M8OI decreased H_2O_2 release (through the inhibition of reverse electron flow). These data are indicative of a complex I inhibitor. M8OI has been identified in the environment and data suggests that it is acutely toxic, likely through the inhibition of oxidative phosphorylation. Therefore M8OI (and potentially other structurally related ionic solvents) may be toxic via an interaction with complex I in the mitochondria of sensitive cells.

PS 1222 Physiologic and Metabolic Impact of Persistent Organic Pollutants on Gut Microbiota

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Emerging evidence supports that exposure to persistent organic pollutants (POPs) can impact gut microbiota-host metabolic homeostasis. Here, we examined the direct effects of POPs including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and polychlorinated biphenyls (PCB-126 and PCB-153) on the microbiota using *in vitro* models. Mouse cecal microbiota and seven individual bacteria species were incubated with two doses of TCDD (high: 1 μM and low: 0.1 μM), TCDF (high: 10 μM and low: 1 μM), and PCBs (high: 10 μM and low: 1 μM) in an anaerobic chamber. NMR- and mass spectrometry-based metabolomics combined with flow cytometry and growth rate measurements (OD_{600}) were used to systematically profile the direct impact of TCDD, TCDF, and PCBs on the microbiome. This study demonstrated that (1) 4 h incubation of TCDD, TCDF, and PCBs resulted in significant decreases in cecal microbial metabolic activity in a dose-dependent manner (carboxyfluorescein diacetate stained bacteria decreased from 65.9 ± 5.2% [vehicle] to 37.5 ± 5.4% [TCDD H], 38.8 ± 4.5% [TCDF H], 29.5 ± 5.6% [PCB-126 H], and 42.7 ± 3.7% [PCB-153 H]); (2) 4h exposure of TCDD, TCDF, or PCB directly and rapidly affect cecal bacterial global metabolism including disrupted amino acid, nucleotide, lipids, and carbohydrate metabolism (e.g., branched-chain amino acids decreased to 0.91-fold [TCDD H], 0.97-fold [TCDF H], 0.96-fold [PCB-126 H], and 0.90-fold [PCB-153 H]; xanthine decreased to 0.65-fold [TCDD H], 0.77-fold [TCDF H], 0.76-fold [PCB-126 H], and 0.64-fold [PCB-153 H]; lipid increased 1.10-fold [TCDD H], 1.16-fold [TCDF H], 1.17-fold [PCB-126 H], and 1.18-fold [PCB-153 H]); (3) individual bacteria species showed extreme variation in response to TCDD, TCDF, or PCB exposure; (4) the membrane destructive effect of (non-coplanar) PCB-153 is more dramatic than (coplanar) PCB-126 on microbiota. These data provide new insights into the direct role of POPs on the gut microbiota and begin to establish possible microbial toxicity endpoints which may help to better inform risk assessment.

PS 1223 Multigenerational Impacts of Dietary Exposure to the Flame Retardant BDE-99 in the Atlantic Killifish (*Fundulus heteroclitus*)

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Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants that are persistent, bioaccumulative, and ubiquitous environmental pollutants linked to a variety of adverse health effects, including endocrine disruption and developmental neurotoxicity. Although maternal transfer of PBDEs has been documented, relatively few studies have investigated effects propagated across generations. To first characterize physiological and molecular outcomes in the exposed generation, we assessed the effects of dietary exposure to a predominant PBDE congener, BDE-99, in the Atlantic killifish (*Fundulus heteroclitus*). Adult wild-caught killifish were fed diets amended with a range of concentrations of BDE-99 for 38 days. Bioaccumulation was assessed, and measured internal dose ranged from 2.4 to 35.9 μg/g fish dw by termination of the exposure. Fish length, weight, and fecundity were measured at three time points throughout the exposure period. On days 10 and 39, subsets of fish were sampled, and tissues (gonads, liver, abdominal fat, and brains) were weighed and archived for molecular analyses. Energetic reserves (fat and liver mass) were affected and reproduction significantly impaired by all concentrations of BDE-99 compared to control. Next, to evaluate multigenerational impacts, adults were again exposed through diet to BDE-99 for 64 days. Target BDE-99 concentrations were 1 and 4 μg/g fish dw to achieve doses similar to and lower than the prior exposure. Fish were manually strip spawned at five time points and eggs fertilized to produce the F1 generation. At termination of the dietary exposure, the F0 adults were sampled, terminal measurements taken, and tissues archived for molecular analyses. No significant differences in growth, tissue indices, reproduction, or fertilization rate were observed between treatments. Chemical analyses are ongoing to quantify body burden and maternal transfer, but preliminary results suggest that a similar bioaccumulation rate was achieved with both exposures. F1 larval behavior was assessed at 3 and 14 days post-hatch by analysis of activity during alternating light/dark conditions. Ongoing experiments are testing for both short-term and persistent behavioral alterations in the F1 generation, as well as impacts propagated across generations from these parental exposures.

PS 1224 Developmental Exposure to Polychlorinated Biphenyls (PCBs) Causes Voiding Dysfunction in Young Adult Mice

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Lower urinary tract symptoms (LUTS), which can affect individuals of all ages, severely reduce quality of life. Despite their prevalence, risk factors and mechanisms responsible for LUTS onset and progression are poorly defined. PCBs remain a current and significant environmental risk to human health, yet whether PCBs impact the developing bladder and contribute to LUTS risk is unknown. We tested the hypothesis that developmental exposure to PCBs alters voiding function in young adult male and female mice. Wild type mice were exposed throughout gestation and lactation to 0, 0.1, 1 or 6 mg/kg/d PCB mix via the maternal diet. The PCB mix proportionally mimics the 12 most abundant PCB congeners in serum of women at increased risk of having a child with a neurodevelopmental disorder. Voiding function was tested in male and female offspring at ~6 weeks of age using three different methods; the spontaneous void spot assay, uroflowmetry, and anesthetized cystometry. These methods allow for quantification of voiding function in awake freely moving mice and anesthetized mice. Effects of PCBs on voiding function were sex- and dose-dependent. Preliminary results of the void spot assay revealed a significant increase in the number of small urine spots (0-0.1cm) produced by male mice in the 0.1 and 1 mg/kg/d PCB group vs. vehicle control, while this increase was only seen in female mice in the 6mg/kg/d PCB group. Uroflowmetry identified a significant decrease in strength of urine stream in male mice in the 0.1 mg/kg/d PCB group vs. vehicle control. Anesthetized cystometry revealed a significant decrease in time between voids, regardless of sex, in the 0.1 and 6 mg/kg/d PCB group vs. vehicle control. Together, these results indicate that males are more sensitive than females to developmental PCB effects on voiding; PCB exposure produces more frequent voids of small volume; effects of PCBs on voiding are not completely behavioral since anesthetized animals display voiding dysfunction. Overall, these results indicate

that developmental PCB exposure affects voiding function and further support the hypothesis that urinary function is influenced by early-life exposure to environmental pollutants. *Supported by (K99 ES029537 KPK; U54 DK104310 DEB; R01 ES014901, P01 ES011269 PJL).*

1225 Dioxin and Furan Congener Contributions to Blood TEQ in the Current NHANES Database

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Dioxins and furans are primarily produced as industrial byproducts, while PCBs are typically associated with historical use of Aroclors. Certain PCB congeners are assumed to possess "dioxin-like" potencies, and these congeners and the dioxin/furans comprise the blood total toxic equivalency (TEQ) for any given individual. The purpose of this analysis was to use the National Health and Nutrition Examination Study (NHANES) data to assess the most current (2005-2010) congener-specific percent contribution to blood TEQ in the general US population and in various demographic groups. In the general population, the primary contributors to blood TEQ were 12378PeCDD (32%), 123678HxCDD (16%), PCB-126 (13%), 23478PeCDF (11%), and 2378TCDD (9%). The remaining TEQ was made up of 13 dioxin/furan and 10 PCB congeners, contributing 0-3% each. In general, very similar patterns were observed in all demographic groups, and the congener-specific percent contribution to TEQ appears to be relatively stable throughout the 2005-2010 sampling period. However, there were some exceptions, for example the percent contribution of PCB-126 in black males and females 40-59 years old increased from 2005-2006 to 2009-2010, and TCDD in white males 12-19 and 20-39 years old appears to be increasing over that time period as well. It is worth noting that the blood TEQ patterns observed in NHANES are generally consistent with those reported in various beef and pork products consumed in the US The point sources for the primary contributors 12378PeCDD and 123678HxCDD are not well understood, but the half-lives of these congeners are estimated to be 11.2 and 13.1 years, respectively (vs 7.2 years for 2378-TCDD), which likely explains their relatively high body burdens in humans and livestock. Interestingly, similar blood TEQ patterns to those reported here have also been reported in the literature in individuals purportedly exposed to elevated levels of TCDD in contaminated environmental sources.

1226 Strategizing Read-Across in Toxicological Risk Assessments of Medical Devices Using Potency and Severity Indices from Data-Rich Toxicological Substances

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Recent changes in ISO 10993-1:2018 and the 2016 FDA Guidance regarding ISO 10993-1 have made chemical characterization necessary for most medical devices. Extractions of medical devices in the recommended solvents and use of advanced analytical instrumentation have resulted in identification of numerous chemicals lacking sufficient toxicological data for risk assessment. Read-across is a commonly used technique that predicts hazard in the absence of empirical data. However, it is an evolving approach with several open issues, including a lack of consensus regarding the extent and type of evidence necessary to support its use. This study established a standardized workflow of using a read-across tool along with the potency and severity of harm indices in a large data set of commonly extracted chemicals from medical devices. To determine the feasibility of effective grouping of medical device chemicals for use of read-across, a publicly available software tool, ChemACE (Chemical Assessment Clustering Engine), was applied across a large chemical inventory from the ECHA database to cluster chemicals based on structural features. Points of departure (POD) values were identified from multiple endpoint toxicity studies of high reliability score (Klimisch score of 1 or 2) for these chemicals. Per ISO/TC 194/WG 11, potency and severity of harm categories were determined for these chemicals, and trends were examined. Although the PODs of chemicals within clustered groups exhibited a considerably wide range, similar potency and severity categories were observed within the clusters. Overall, results show that when chemical clustering predictions were analyzed along with the experimental data from well-conducted toxicological studies, read-across may be a viable option for filling in data gaps for data poor chemicals. However, consideration of strategies such as use of uncertainty factors may be justified when extrapolating toxicological data from data rich group members to other group members lacking toxicological data.

1227 Risk Assessment of Extractables from Warmed and Room Temperature Dental Composite Restoratives

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Dental composite restoratives are critical to the oral health of patients worldwide. In the past decade, dental composite warmers that can heat composites up to 70°C have been suggested to improve product flowability and adaption with minimal heat transfer to the pulp. The purpose of this study was to investigate the effects of composite warming on product extractables and biocompatibility. Triplicate samples of two dental composites, 3M ESPE Filtek Universal Restorative (FUR) and 3M ESPE Filtek Supreme Ultra Flowable Restorative (FSUF), were subjected to a warming procedure consisting of either a single 1-hour heating event (70°C) for single-use capsule packaging (FUR) or 20 repeated 1-hour cycles of heating to 70°C and cooling to room temperature for reusable syringe packaging (FSUF). The three warmed samples of each product and three room temperature samples (RT) of each product were cured according to manufacturer's instructions and extracted according to ISO 10993-12 at 37°C for up to 35 days (exhaustion) in methanol, heptane, and 5% ethanol. In each case, 1) no new extractables were identified in the warmed samples relative to the RT samples, 2) most extractables were released from the warmed samples at similar or decreased concentrations relative to the RT samples, and 3) extractables with increased concentrations from the warmed samples relative to the RT samples were released at amounts that compared favorably to toxicological thresholds (e.g., ICH M7). These results indicated that the warmed and RT samples of each product were toxicologically equivalent according to ISO 10993-18: 2005. Based on a detailed risk assessment of the extractables, FUR in capsules and FSUF in syringes are safe for their intended use when warmed to 70°C for up to 1 hour prior to application. This risk assessment approach may be applicable to the biocompatibility evaluation of other dental composite restoratives prepared under warmed conditions.

1228 Establishing a Tolerable Intake for Dimethyl 4-Toluidine through Use of Benchmark Dose Modeling

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As specified in ISO 10993-17:2002, "The determination of the suitability of a medical device for a particular use involves balancing any identified risks with the clinical benefit to the patient associated with its use. Among the risks to be considered are those arising from exposure to leachable substances arising from medical devices." Dimethyl 4-toluidine is used to make acrylic resins and is used in the cement in most hip and bone replacements. After dimethyl 4-toluidine was detected during extraction testing of a medical device, a risk assessment was performed to calculate a tolerable intake (TI). A TI is defined as the estimate of the average daily intake of a substance over a specified time period that is considered to be without appreciable harm to health. Dimethyl 4-toluidine is a potential human carcinogen based on chronic oral studies in rodents conducted by the National Toxicology Program. Increased tumor incidences were reported at multiple sites, including liver, nose, thyroid, and/or lung in rats and mice. We selected hepatocellular carcinomas in male mice to derive a cancer slope factor (CSF) for dimethyl 4-toluidine as this tumor type had the highest incidence and was detected across both species and sexes. The resulting CSF should be protective of other tumor types. Duration-adjusted doses and liver tumor incidences were fit to the multistage cancer model using US EPA Benchmark Dose Software (BMDS) with a 10% increase in tumor incidence as the benchmark response. Relevant fit parameters and a visual curve inspection indicated that the model fit adequately. The resulting animal CSF of 0.0257 $(mg/kg/day)^{-1}$ was scaled to a human-equivalent CSF by multiplying by the $(human\ body\ weight/animal\ body\ weight)^{1/4}$ ratio; this yielded a human CSF of 0.1579 $(mg/kg/day)^{-1}$. The TI is derived by dividing an acceptable cancer risk level by the human CSF, which can be adjusted by less-than-lifetime exposure for medical devices with shorter patient contact. While ISO 10993-17:2002 specifies an acceptable cancer risk level of 10^{-4}, ICH M7 Guidance and US FDA use an acceptable risk level of 1 in 10^{-5}, which represents the potential for 1 in 100,000 people to develop cancer following a lifetime of daily exposure. Using a cancer risk value of 10^{-5} and 0.1579 $(mg/kg/day)^{-1}$ CSF results in a 0.0633 µg/kg/day TI for dimethyl 4-toluidine extracting from permanent contact devices. This TI can be compared to expected exposure levels to dimethyl 4-toluidine resulting from use of a specific medical device.

1229 Toxicological Risk Assessment of Simulated Isopropyl Alcohol (IPA) Exposure Dose Estimates in Neonates

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IPA-filled caps are single-use devices attached to luer access values to prevent central line-associated blood stream infections. Sauron et al. (2015) simulated clinical use of IPA caps in neonatal intensive care units (NICUs) neonates and reported altered appearance of luer valves, as well as excess IPA quantity in simulant, and posited IPA caps should not be used for neonates without additional research. Analytical chemistry and toxicological risk assessment methods used by Sauron et al. were not reported in sufficient detail to assess data reliability and confidence in the findings. We conducted an independent study of the impact of IPA caps on luer valves by performing worst-case *ex vivo* simulations of IPA cap use in neonates. Results from a survey of NICUs were used to plan our simulation to identify the worst-case scenario. To simulate clinical use, a circuit was created consisting of a SmartSite luer valve, SwabCap, Y-connector with crimp, and a glass collection vial with a septum cap. Worst-case clinical conditions included (a) incubating luer valves with IPA caps at 37ºC, (b) simulating 20 drug infusions that occur at four periods over a day, (c) swiping proximal end of the luer value with an IPA pad, and (d) simulating drug administration using water (simulant) that included a prewash (0.5 mL), drug infusion (0.3 mL), and post-wash (0.5mL). We also used the standard patient care of swiping valves with IPA pads after removal, and before subsequent attachment, of IPA-filled caps. The quantity of IPA in infusates was then determined by gas chromatography with flame ionization detector. Simulating worst-case number of drug infusions in a single day resulted in IPA concentrations of 32.33 mg/dL IPA (with IPA cap) and 2.37 mg/dL (swiping only). The *ex vivo* worst-case use of IPA impregnated caps in neonates resulted in IPA quantities that exceeded the blood toxicity threshold for children (25 mg/dL); whereas, swiping luer access with an alcohol pad alone did not. In conclusion, the result of our *ex vivo* simulation studies provide additional evidence that an excess amount of IPA could be unintentionally administered into the bloodstream of neonates hospitalized in NICUS. Furthering our understanding of how an excessive amount of IPA could be administered to neonates is needed.

1230 A New Approach to Assessing Toxicity of Detergent Residuals for Reusable Device Cleaning Validations

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The accurate validation of cleaning procedures for reusable medical devices is a critical step in protecting patient safety. As part of the validation process, many residual endpoints are often addressed, as recommended in AAMI TIR30. Among these endpoints is detergent residual testing to verify that detergents used in the cleaning process do not remain on the medical device in cytotoxic concentrations after the cleaning procedure is complete. AAMI TIR30 suggests that the toxicity of detergent residuals may be addressed using the cytotoxicity testing methods outlined in ISO 10993-5. However, because ISO 10993-5 is designed to address the cytotoxicity of the medical device materials and manufacturing processes, this suggestion often leads to the inclusion of an exaggerated extraction procedure in serum supplemented cell culture media which is intended to extract all potentially cytotoxic chemicals from the device materials. This can result in an overly stringent test method for assessing the effect of residual detergent. Although extractions in cell culture media are frequently performed for biocompatibility assessments, ISO 10993-5 allows for other extraction conditions which simulate the extraction that occurs during clinical use if they provide an adequate measure of the hazard potential. Herein, we present an alternative approach to assessing the toxicity of detergent residuals for use in validating cleaning procedures of reusable medical devices. This approach utilizes water based extractions commonly used for other cleaning validation residual marker tests (total organic carbon, hemoglobin, protein, etc.) to assess detergent residuals in a more clinically relevant manner. Using quantitative cytotoxicity testing - which measures the metabolic activity of cells exposed to the detergent solution to calculate a percent viability value - we are able to construct dose response curves for commonly used detergents. This allows us to determine the concentration of residual detergent on a device following a cleaning procedure and provides detailed data that can be used to efficiently assess the associated toxicological risk.

1231 A Flow Cytometry Method for Characterizing Platelet Activation

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Hemocompatibility testing is critical for assessing the safety of blood-contacting medical devices. Comprehensive hemocompatibility testing requires examining a wide range of possible adverse effects cause by direct or indirect blood contact, such as hemolysis, complement activation, and thrombus formation. Moreover, these domains each encompass complex intercellular processes with many potential targets for analysis. For example, the current testing paradigm of platelet function may involve exposing the device to human whole blood and performing simple blood counts and/or macroscopic evaluation to determine the extent of platelet activation and clot formation as described in ASTM F2888-19. However, this approach does not capture any observations for device-mediated initiation of any steps in the platelet activation sequence prior to aggregation. We have validated a method to evaluate platelet activation by quantifying surface p-selectin expression after exposure to various materials. This method will provide an additional level of detail about potential platelet activating properties of a medical device. Flow cytometry has been used previously to measure platelet activation for clinical and research purposes. We sought to adapt this method to test for platelet activation induced by exposure of blood to medical devices or materials. We determined that processing fresh whole blood to platelet-rich plasma (PRP) by gentle centrifugation enhanced the signal compared to fresh blood itself. In each experiment, devices were exposed to PRP according to an extraction ratio of 6 cm^2/mL for 1 hour. A blank control consisting of untreated PRP, and a positive control consisting of ADP, a potent agonist, were also used. After the exposure, excess plasma was removed from the articles and combined with anti-CD61 (to stain for platelets) and anti-CD62P (to stain for activated platelets) antibodies. Flow cytometry was then performed to quantify the percentage of CD62P+ over the total CD61+ cells to measure the percentage of activated platelets. In order to optimize the method, we investigated the effect of several experimental factors, including anticoagulant usage, donor variability, and selection of reference materials to serve as controls. Our results indicate that the flow cytometry-based method is consistent and reproducible, easy to perform with short time to results, and well-correlated with results from the standard platelet and leukocyte count assay. The flow cytometry-based platelet activation method is a powerful supplement to the standard medical device hemocompatibility testing.

1232 Quantification of Aluminum Release in a Smiths Medical Intravenous Fluid Administration Set via ICP-MS

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Recent attention has been drawn to the tendency of aluminum-plate intravenous fluid warming devices to release concentrations of aluminum into the administered fluid. The risk of aluminum leaching into warmed fluids delivered via Massive Transfusion Protocols in emergency care during trauma, organ transplantation, vascular emergencies and postpartum hemorrhage was explored in a complex simulated-use study outside of traditional ISO 10933 conditions to better elucidate aluminum exposure levels consistent with clinical use. We designed a study to quantify aluminum release from the Level 1® Normothermic I.V. Fluid Administration Set with an uncoated aluminum-plate warming system in three clinically simulative solutions: 0.9% sodium chloride, Lactated Ringer's Solution, and bovine whole blood. The disposable-hardware system was evaluated during one hour of use at 42°C and a clinically relevant flow rate of 30 mL/min. Samples from the effluent were obtained at regular intervals and were analyzed via ICP-MS, revealing different aluminum release profiles depending on the solution. Exposed to 0.9% sodium chloride, the concentration of aluminum in solution gradually increased from undetectable to 6.90 ng/mL. When Lactated Ringer's Solution was used, a consistently increasing aluminum release was measured over time starting at 74.6 ng/mL at 10 minutes and ending the one hour period at 277 ng/mL. An erratic profile of release into bovine whole blood was detected, varying from 434 ng/mL to 75.0 ng/mL, with the rate of aluminum leaching generally decreasing over time. Samples of cumulative aluminum leaching revealed negligible overall release into 0.9% normal saline and bovine whole blood over the entire course of the assessment (one hour), with a release of 98.1 ng/mL into Lactated Ringer's Solution. Our findings indicate that the evaluation of devices under simulated use utilizing clinically-relevant fluids and conditions can enhance the evaluation of device safety by providing a realistic exposure assessment.

1233 Chemical Analysis of Medical Device Materials to Support Equivalency Determination

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Biological evaluation of medical devices often includes chemical characterization (as described in ISO 10993-18). Device materials are subject to extraction where by materials, processing additives, residues, as well as material breakdown products may be released. These extractables have potential consequence on the biological response to the device during its use. Chemical characterization studies are followed up by toxicological risk assessments which evaluates potential harm from exposure to the extractables. In some cases, evaluation of material equivalence of similar device materials is required. Following extraction, chemical analysis is performed by various methods, such as GC/FID/MS, FTIR, and LC/UV/MS. Current technology in high resolution mass spectrometry may improve these analyses. This presentation discusses development of improved data analysis methods to probe chemical analysis data from two materials' equivalency. A panel of medical device materials all composed of polypropylene were analyzed. Multiple rounds of extractions (24 hour at 50°C 200 rpm) were used to obtain non-volatile residue. (NVR) Test extracts were introduced to different instruments: a GC/FID/MS (7890B/5977B Agilent) equipped with an internal splitter enabled FID and MS signal to be acquired simultaneously. An UHPLC-QTOF-MS system (6540 UHD- Agilent). Amounts of extractables were estimated semi quantitatively by comparing to a set of internal standards. FTIR data is also obtained for NVRs. Standards of various polymer additives were prepared and analyzed by LC-UV-MS system where the UV absorbance and MS data were acquired together. Processed and raw polypropylene materials were extracted by isopropanol and hexane. GC/FID/MS analysis are acquired for every sample to add additional dimension of data. The GC/MS data were also processed for identification of analytes using the 2017 NIST Mass Spectral Library. Preliminary results showed more than 20 compounds tentatively identified. Ongoing work includes the use of Q-TOF MS with greater resolution (~40,000) for unknown identification using a commercial E&L Database as well as open access databases. A scoring scheme for binary comparison of the data was utilized using Principal Component Analysis.

1234 Using Physics-Based Models to Provide Conservative, Clinically-Relevant Estimates of Patient Exposure to Extractables from Medical Device Polymers

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Biocompatibility assessment of new medical devices remains a challenging and resource-intensive burden to all types of FDA submissions. Extraction testing, outlined in ISO-10993, prescribes conditions for identifying and quantifying compounds that may be released from medical devices. Our objective is to harness data from common extraction techniques in a diffusion-limited transport model to forecast a worst-case, clinically relevant exposure. For devices of a given geometry with stable physicochemical properties *in vivo* and homogenous and dilute additive content, exposure may be estimated based on total additive mass and a diffusion coefficient (D_p) for the additive within the matrix material. The latter may be conservatively provided by an upper bound based on literature data for relevant polymer matrices and the additive molecular weight. Samples were produced via twin-screw extrusion and injection-molding from materials including high-density polyethylene (HDPE) containing 1 wt% additive chosen from common antioxidants including β-hydroxy toluene (BHT; Mw 220 Da), Irganox 1076 (Mw 531 Da) and 1010 (Mw 1178 Da). Extraction testing was performed in accordance with ISO10993-12 under exaggerated conditions, using polar (H2O), mid-polar (IPA), and non-polar (hexane) solvents at 50°C. Samples were analyzed via liquid chromatography mass spectrometry (LC-MS) for the target additive masses to determine release rates, which were used to calculate diffusion (D_e) and partition (K) coefficients for each additive-solvent combination. D and K values were used to assess the suitability of common extraction conditions to recover total additive mass needed for the exposure model. Extraction results from HDPE samples consistently indicated greater diffusion rates (D_e) relative to model predictions (D_p) - $4.7x10^{-8}$ vs $1.2x10^{-9}$ cm^2/s for BHT; $1.5x10^{-8}$ vs $5.8x10^{-11}$ for 1076; $1.8x10^{-9}$ vs $2.2x10^{-12}$ for 1010. Consequently, extraction studies implied greater exposure rates than predicted by the model - 235 vs 65 µg/day for BHT; 216 vs 14.6 µg/day for 1076; 131 vs 2.9 µg/day for 1010. Samples swelled significantly (~1 vol% in IPA, ~15 vol% in Hexane), contributing to their increased diffusion and release relative to model predictions. Results suggest physics-based models may provide an improved approach to assessing worst-case, clinically relevant exposure within a toxicological risk assessment framework.

1235 A Critical Evaluation of the NAVI Model for Thrombogenicity Evaluation of Cardiovascular Medical Devices: Correlation between *In Vitro* Hemocompatibility Test Results and *In Vivo* Thrombus Scores

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According to ISO 10993-4: 2017, direct blood-contacting medical devices should be evaluated for thrombogenic potential, and the *in vivo* 4hr non-anticoagulated venous implant (NAVI) model is a standard test procedure used to assess acute thrombogenicity. However, limitations in test methodology such as inherent differences in individual animal thrombogenic reactivity, lack of statistical power, placement in venous anatomy, incubation period, and lack of anticoagulants may limit the NAVI model's predictiveness of the thrombogenic potential of a medical device under clinical-use conditions. ISO 10993-4:2017 also indicates that the manufacturer may decide if *in vitro* tests are an appropriate alternative to *in vivo* testing. Common *in vitro* tests are used to assess coagulation [partial thromboplastin time (PTT)], platelet counts (% loss), and complement (SC5b-9) activation. To determine if there is a correlation between *in vitro* test results and elevated *in vivo* thrombus scores, the results of *in vivo* thrombogenicity studies were compared to corresponding *in vitro* data (PTT, platelet counts and SC5b-9 complement activation) in the same devices. Results from 15 NAVI studies showed thrombus scores were variable among individual animals (i.e. scores differed by 2 among three animals in half of the dataset). In this analysis, studies were divided into two groups: thrombogenic group with scores of ≥3 in at least 2 animals; less thrombogenic group with scores of ≤ 2 in at least 2 animals. Although no association between platelet counts and elevated thrombus scores was observed, PTT results (clotting time normalized to plasma) were on average 20% lower, and SC5b-9 levels were statistically significantly higher in the thrombogenic group. These results suggest reduced PTT and increased SC5b-9 may be associated with *in vivo* thrombogenicity in the NAVI model. Historically, platelet counts have been assessed using sodium citrate anticoagulation. Platelet counts utilizing heparinized human blood per ASTM F2888:2019 may improve the correlation. In conclusion, *in vitro* hemocompatibility tests, possibly combined with data from a dynamic *in vitro* flow loop may provide a feasible and appropriate alternative to *in vivo* thrombogenicity testing.

1236 Cytotoxicity and Mutagenicity Assessment of Lithium Phenyl(2,4,6-trimethylbenzoyl) phosphinate (LAP) Photoinitiator with Exposure to 405 nm Light

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The use of photosensitive resins in biomedical applications exposes embedded cells and/or surrounding tissue to stressors such as chemicals, light, and heat, which might lead to cell injury. Photosensitive resins are made reactive to light by addition of a photoinitiator; concurrent exposure of the photoinitiator to a compatible light wavelength initiates a polymerization reaction that exposes cells to free radicals, which can be cytotoxic, genotoxic, and mutagenic. LAP is a photoinitiator popular in bioprinting due to its water solubility and compatibility with visible (405 nm) light but is a lithium salt that can accumulate in renal collecting duct cells causing loss of urine concentrating ability or cell injury. This project investigates the effect of LAP exposure on cytotoxicity in M-1 murine collecting duct cells and concurrent exposure of LAP and 405 nm light on mutagenicity in TA98 and TA 100 *S. typhimurium*. Confluent M-1 monolayers with observed "domes" indicative of active water transport were exposed to LAP concentrations ranging from 1 to 20 mg/mL or the corresponding equimolar concentration of lithium chloride for 24 hr; cadmium chloride (1 mM) was used as the positive control. No significant decrease in viability was observed after exposure to 5 mg/mL LAP using the Neutral Red or Alamar Blue assays; viability decreased to 49% and 70% with exposure to 7.5 mg/mL. Exposure to 10 mg/mL or above resulted in low viability indistinguishable from the positive cytotoxicity control. For the bacterial mutagenicity assay, no increase in revertant colonies above the background was observed in TA100 exposed to LAP concentrations up to 10 mg/mL with 15 minutes of 3 mW/cm2 405 nm light from an LED source, an exposure previously determined to exaggerate conditions during bioprinting. Exaggerated exposure of 15 mg/mL LAP with 1 or 5 min of 224 mW/cm2

light exposure to TA100, TA98, or WP2uvrA also showed no mutagenic effect; however, a cytotoxic response was observed with 5 min light exposure. UV-C light, 4-Nitroquinoline-1-oxide for TA100 and TA98, and 2-nitrofluorene for WP2uvrA were used as positive mutagenicity controls. Typical LAP concentrations used in bioprinting (<5 mg/mL) were not cytotoxic to confluent M-1 cell layers and exposures, even under exaggerated conditions, using the Ames assay to LAP and 405 nm light showed no evidence of mutagenicity.

1237 The *In Vitro* Toxicity of a Dental Resin Monomer Varies among Different Cell Culture Models

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In vitro cytotoxicity tests are routinely carried out during biocompatibility evaluation of medical devices. By using immortalized cell lines, such tests become relatively simple and fast to perform. However, a wide range of cell lines is available and various protocols describe the use of specific cell types without a thorough justification. Cell lines that are commonly used for toxicity studies differ both in source (species and organ) and how they have become immortalized (e.g. cancer cells and virus transfected cells). Based on this, it can be assumed that they differ in many properties, including their capacity to handle different exposure scenarios. Hence, we hypothesize that results obtained by *in vitro* cytotoxicity tests depend strongly on the chosen cell line. In this study, we have compared how four commonly used cell lines respond to 2-hydroxyethylmethacrylate (HEMA) exposure. 1) A549 cells (human, alveolar carcinoma), 2) BEAS-2B cells (human normal bronchial epithelium, SV40 transformed), 3) RAW 264.7 cells (murine, macrophage-like, Abelson leukemia virus transformed) and 4) L929 cells (murine, fibro-sarcoma). All cell lines were exposed to 1-8 mM HEMA (a methacrylate monomer that both dental personnel and dental patients are exposed to during treatment). The endpoints used to measure cellular responses to HEMA were 1) viability (MTT assay), 2) cell death analyses (fluorescence microscopy of Hoechst/propidium iodide stained cells) and 3) cell growth pattern (flow cytometry of DAPI-stained cells). The relative viability (% viable cells compared to unexposed cell culture) after 24 h HEMA exposure (8 mM) varied from 86 % (L929 cells) to 32 % (RAW 264.7 cells). Similarly, the portion of dead cells varied from 4% (L929 cells) to 72 % (RAW 264.7 cells). HEMA also caused altered cell growth pattern in some of the cell lines. In A549 cells, an increased portion of cells in S-phase was measured, while a decreased portion of cells in S-phase was observed in RAW264.7 cells. No significant difference was measured in exposed BEAS-2B and L929 cells. In summary, our results show that the outcome of an *in vitro* cytotoxicity test may depend on the cell line used.

1238 Thrombogenicity Testing Results for Legally Marketed Comparator Devices (LMCD): Comparison between the Traditional NAVI Assay and an *In Vitro* Ovine Blood Loop

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ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory requirements for approval of blood-contacting medical devices. We are continuing to develop an *in vitro* thrombogenicity assay using minimally heparinized ovine blood that has been successfully used in lieu of the non-anticoagulated venous implant (NAVI) assay in recent submissions with the US FDA. Our blood loop assay is primarily used for testing thrombogenicity of test articles (catheter-shaped devices typically less than 18 Fr) and comparing them to similar predicate devices. We also frequently perform the traditional NAVI model in canines. The NAVI test requires 2-3 naïve animals, typically large dogs, and also compares the performance of a test article (a new product or new manufacturing procedures/materials in development) to a predicate. In both the blood loop and the NAVI assays, predicate devices are typically legally marketed comparator devices (LCMD). One observation that is rarely discussed are the high thrombogenicity scores of LCMD's in the NAVI assays. Ideally it is expected that the test article will have an equal or lower relative thrombus score than the LCMD. Using the ISO 10993-4, Table C.2 scoring scheme, a score of 2 or lower (<50% of surface covered with thrombus) is associated with minimal to no risk for thrombogenicity in clinical use. If the LCMD scores a 3 or higher (>50% of surface covered with thrombus), the interpretation of the test becomes problematic even if the test article is equivalent to the LCMD. In this retrospective analysis, we have compiled thrombogenicity scores of control/predicate devices (limited to assays which used LMCD's), both the discrete score from the classification standard scoring scheme and the continuous values obtained from the percent surface area associated with thrombus. We have compared results from >75 NAVI studies and >50 *in vitro* blood loop studies. These compiled results show ~25% of LMCDs score >3 (>50% of the surface covered in thrombus) in the NAVI model while <5% of LMCDs score >3 (>50% thrombus) in the Blood-Loop assay. In addition, the median score and mean % thrombus for LMCD in the blood loop assay is substantially lower that the median and mean scores for LMCD in the NAVI assay. This retrospective assessment highlights a high proportion of false positive scores for LMCD in a large number of NAVI assays. This is an alarming observation; that a standardized assay so frequently yields scores for LMCD's in a range that would predict a risk for clinical use (>50% of material surface covered with thrombus). Overall, these results are strong supportive evidence for the superiority of the Blood Loop assay over the *in vivo* alternative NAVI.

1239 Dose- and Time-Dependent Formation of γ-H2AX, a Biomarker for Early Detection of Bladder Carcinogens, and Its Potential Role in Tumorigenesis in the Rat Urinary Bladder

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We previously reported that immunostaining for γ-H2AX, a biomarker of DNA damage, in the rat urinary bladder is useful for early detection of bladder carcinogens in 28-day toxicity studies. Here, we examined the dose and time dependency of γ-H2AX formation and its role in tumorigenesis in the rat urinary bladder. In experiment 1, 6-week-old male F344 rats were orally administered *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN; 0%, 0.0001%, 0.001%, 0.01%, 0.02%, or 0.05% in drinking water), a genotoxic bladder carcinogen, and melamine (0%, 0.3%, 1.0%, or 3.0% in the diet), a nongenotoxic bladder carcinogen, for 2 days or 4 weeks. Immunohistochemical analysis demonstrated that γ-H2AX-positive epithelial cells in the bladder mucosa were significantly increased in a dose-dependent manner in the BBN and melamine groups. The ratios of γ-H2AX-positive cells at week 4 in the BBN and melamine groups were higher than those on day 2, demonstrating a time-dependent increase in γ-H2AX formation. Immunofluorescence staining showed that although γ-H2AX formation and Ki67 expression were colocalized in bladder epithelial cells of rats in the melamine groups, many γ-H2AX single-positive cells without Ki67 expression were detected in the BBN groups, suggesting that the associations of genotoxic mechanisms induced by chemicals could be determined by analyzing the colocalization of γ-H2AX and Ki67. In experiment 2, 6-week-old male F344 rats were administered 500 ppm BBN for 4 weeks and necropsied at 0, 2, 4, 8, 16, or 32 weeks. Although diffuse simple hyperplasia found at week 0 was recovered to the normal-like urothelium until week 4, newly developed focal proliferative lesions, such as focal simple and papillary/nodular hyperplasia, were increased after week 4 and progressed to papilloma and carcinoma in a time-dependent manner. γ-H2AX-positive cells persisted at significant high levels not only in focal proliferative lesions but also in the normal-like urothelium, even after withdrawal of BBN. These results suggested that γ-H2AX formation was increased in a clear dose- and time-dependent manner. In addition, γ-H2AX could be a useful biomarker associated with the development of bladder tumors rather than a temporal response to chemicals.

1240 Characterization of Casting-Induced Muscle Injury and Inflammation in a Mouse Model

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The mouse casted limb (immobilization) model induces skeletal muscle atrophy and may be used to study drug efficacy for recovery from atrophic conditions. In this model, one hind limb is casted to induce atrophy and the contralateral uncasted limb is typically used as an unaffected comparator. Uncasted mice (shams) are often included for reference. Two studies conducted in female C57BL/6J mice employed 2 weeks of casting followed by 1 week of uncasted recovery, with cohorts necropsied at interim time points during the casting and recovery phases. In study 1, skeletal muscle degeneration biomarkers [skeletal troponin I (sTnI) and myosin light chain (Myl3)] and inflammation biomarkers [neutrophil gelatinase-associated lipocalin (NGAL) and tissue inhibitor of metalloproteinase (TIMP1)] were measured in serum and in tissue lysates (from casted and contralateral limbs separately). Increased serum concentrations of sTnI and Myl3 24 and 48 hours after casting with a correlative decrease of these markers in tissue lysates were indicative of muscle degeneration and release of sarcomeric proteins. Although there were small increases in NGAL and TIMP1 during 2 weeks of casting, these inflammation biomarkers in serum and tissue lysates increased markedly and were concurrent with minimal changes in the muscle-related degeneration biomarkers after cast removal, which allowed re-loading of both the casted atrophied limb and the contralateral leg. In study 2, to correlate histologic changes with the serum biomarkers, skeletal muscles (gastrocnemius, quadri-

ceps, biceps femoris) and skin from casted and contralateral limbs were examined microscopically at similar time points explored in study 1. The histologic examination confirmed muscle degeneration and associated inflammation in both the casted and contralateral limbs, but also revealed inflammation related to skin ulceration over the casted leg. The potential impact of muscle degeneration in the non-immobilized limb and cast-related cutaneous ulceration should be considered in the design and interpretation of studies using the casted mouse model.

1241 Elucidating the Gut Microbiome-Metabolome Correlation in Wistar Rats

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The gut microbiome is known to play a huge role in the health and development of the host organism. 'Omics technologies' can provide better understanding of gut microbial functionality. We investigated the correlation between gut microbial communities with changes in the metabolome of plasma, feces and caecum matrices following a 28-day oral treatment with 6 antibiotic (streptomycin, vancomycin, roxithromycin, sparfloxacin, lincomycin and clindamycin) in rats. A predictive functional profiling was developed to predict the metagenomic composition and to associate it with the metabolome profiles. Antibiotics were observed to specifically alter the levels of lipids and fatty acid, bile acid and amino acid metabolism. In particular hippuric acid and indole-3-acetic acid were strongly downregulated. Tested antibiotics had specific influences on the gut flora composition and the two lincosamides show very similar effects, confirming a class-dependent effect. Relative abundances of family-level taxonomy of different treatments were derived. Streptomycin treatment showed similar bacterial abundance as the controls, with Bacteroidetes and Firmicutes as the dominant families. Vancomycin and Sparfloxacin showed reduced Bacteroidetes and Proteobacteria families while the two lincosamides showed increased Firmicutes and Proteobacteria levels. Dose-dependent alterations in Actinobacteria levels was observed in lincomycin treatment. PCoAs of microbiome data showed clustering similar to the PCA of the metabolome data, indicating dependence of various metabolite levels on the gut community. Strong correlations between the gut flora composition (fecal microbiome) with the caecum and feces metabolome profiles of the antibiotic treated rats have been observed. Whereas the correlation with plasma metabolic profiles have been observed to be weaker in comparison. Differential gene regulation with respect to antibiotic treatments was observed and could be associated with the metabolite profiles. Hence, the gut microbial composition but also gene regulation profile could be correlated with the metabolome data.

1242 Mechanistic Modeling Aids in the Interpretation of Alanine Aminotransferase Elevations Associated with Clinical Idiosyncratic Drug-Induced Liver Injury

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The DILIsim Initiative, a public-private partnership, has been developing mechanistic modeling software, DILIsym®, that can use serial assessments of serum alanine aminotransferase (ALT) to estimate % hepatocyte loss and corresponding elevations in serum total bilirubin (TBIL) due to the resulting impaired liver function. The model parameters were optimized to data from acetaminophen overdose patients where observed peak TBIL values were correlated with % hepatocyte loss assessed by liver biopsy (PMID 1214189). Although the modeling has been used in regulatory communications to refine interpretation of Hy's Law cases [i.e. patients experiencing elevations in ALT > 3 X upper limit of normal (ULN) and TBIL> 2 X ULN], the ability of DILIsym to predict peak TBIL values in patients with idiosyncratic drug-induced liver injury (IDILI) has not be tested. An equation P_{ALT}, has recently been proposed to estimate the hepatocyte loss predicted by DILIsym (PMID 30303523). Serial serum ALT values were curve fit (DILIsym v8A) from lab data obtained in clinical drug trials from 39 IDILI patients (n=28 due to anti-tuberculosis treatments and n=11 due to other drugs). P_{ALT} was calculated as: P_{ALT}= ALT_AUC*Peak $ALT^{0.18}/10^5$ $((U/L)^2*h$. The peak TBIL values predicted by DILIsym were significantly correlated with the observed peak TBIL values (r=0.3595; $p<0.05$) and this correlation improved (r=0.7715; $p<0.0001$) as expected with the removal of cholestatic/mixed IDILI cases (R<5, n =3) and cases in which gaps in blood sampling times likely precluded measurement of the true ALT peak (n=5). We next examined the accuracy of the model in predicting Hy's Law cases. TBIL 2 X ULN over or under predictions only occurred in n=3 patients (<10%). Hepatocyte loss predicted by P_{ALT} strongly correlated with the level of hepatocyte loss predicted by DILIsym (r=0.9411; $p<0.0001$). In this study, DILIsym accurately estimated changes in TBIL due to reduction in global liver function in patients experiencing IDILI. Because P_{ALT} also predicts maximum hepatocyte loss, it should be possible to use this open-source tool to predict peak TBIL levels. Because most drugs causing IDILI have characteristic ALT kinetic patterns, P_{ALT} may be useful in clinical trials to predict the peak ALT level that will be observed before functional impairment occurs.

1243 Discovery of Biomarkers to Differentiate Liver Injury and Benign ALT Elevations

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Alanine aminotransferase (ALT) is the standard serum biomarker of liver injury, but ALT elevations can occur without tissue damage. Several drugs and diseases are thought to be associated with increased circulating ALT without cell death. Our goal was to identify biomarkers that can differentiate between true liver injury and benign ALT elevations using both animal models and patients. Methods: In an initial experiment, mice were treated with 100 mg/kg dexamethasone (Dex) to induce benign ALT elevations, 300 mg/kg acetaminophen (APAP) to cause true liver injury, or vehicle. Serum was collected for untargeted proteomics. In a second experiment, mice were treated with the same doses of Dex and vehicle, but a lower dose of APAP (175 mg/kg) to achieve milder ALT elevations similar to the Dex model. Serum was collected to confirm results from the first experiment by immunoblotting. In a third experiment, the biomarkers were measured in plasma and serum from APAP overdose patients without liver injury (peak ALT<100U/L; n=5), overdose patients with mild liver injury (peak ALT 100-1,000 U/L; n=10), and clinical trial subjects with mild ALT elevations thought to be benign based on transience and absence of liver dysfunction (n=3). Finally, rats were subjected to bile-duct ligation (BDL) as a model of cholestasis. Serum ALT was significantly increased in APAP (2,368±223 U/L) and Dex (113±18 U/L) treated mice compared to vehicle groups. Results from qPCR, immunoblotting, and enzyme kinetics confirmed that the ALT elevations in the Dex group were due to increased expression in the liver and muscle, and absence of necrosis in livers from those mice was independently confirmed by two blinded pathologists. Proteomics studies identified 165 proteins detectable mainly in serum from the APAP group, including Aldehyde dehydrogenase 1A1 (ALDH1A1), Alcohol dehydrogenase 1 (ADH1), and Argininosuccinate synthetase 1 (ASS1). Similar results were obtained for all three proteins by immunoblotting when the experiment was repeated with the lower dose of APAP to achieve milder ALT elevations (401±127 U/L), and when comparisons between groups were limited to mice with similar ALT values. The results were further confirmed in the patient samples. Interestingly, none of the proteins were elevated in the rat BDL model of cholestasis. Our data indicate that several novel biomarkers may differentiate between hazardous and benign elevations in circulating ALT and may be specific for hepatocellular injury. *Funded in part by the AASLD Foundation and NIH T32 Grant GM106999.*

1244 Novel Proteomic Biomarkers for the Prediction of Renal Recovery from Dialysis-Dependent AKI Patients

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Acute kidney injury requiring dialysis (AKI-D) is associated with prolonged length of stay, mortality, and progressive chronic kidney disease (CKD) among survivors. Functional renal recovery leading to discontinuation of dialysis is an important clinical outcome. Previous studies have examined few urine or serum biomarkers to predict renal recovery from AKI, however, the performance of biomarkers of renal recovery has not been established. To identify new serum biomarkers that predict renal recovery from AKI-D, study day 8 samples from 76 patients enrolled in the Veteran's Affairs/National Institutes of Health Acute Renal Failure Trial Network study were analyzed by the slow off-rate modified aptamers scan (SOMAscan) proteomic platform to profile 1305 proteins in each sample. Of these patients, 38 recovered kidney function and dialysis was discontinued, while another 38 patients remained on dialysis by day 28. Changes in the serum levels of 81 proteins, 45 of which increased and 36 of which decreased, were detected when comparing samples of patients who were taken off dialysis versus patients who remained on dialysis

by day 28. Serum levels of mortality-associated proteins such as FGF23 and IL-6 were reduced in those that recovered, which was corroborated by Olink analysis. Several kinases related to growth and survival increased in recovered patients, such as tyrosine-protein kinase Fyn, protein Wnt-7a, and Myc proto-oncogene protein, which either regulate glomerular integrity through the Wnt/beta-catenin signaling pathway or regulate angiogenesis, illustrating potential mechanisms of kidney recovery. Receiver operating characteristic (ROC) analysis resulted in AUC of 0.64-0.75 for these kinases and inflammation biomarkers. Thus, this study identifies potential novel predictive biomarkers of renal recovery from AKI-D patients.

1245 Decreased Trf1-Trf2 Negatively Regulates Telomere Length and DNA Damage Foci in Rat Liver Tissue after a High-Fat Diet and Welding Fume Exposure

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Telomeric DNA and shelterin proteins prevent loss of essential genetic information during cell division. Telomere attrition resulting in DNA damage contributes to liver pathology. The telomeric repeat-binding factor 1 (Trf1) and 2 (Trf2), and the protection of telomere 1 (Pot1) are involved in telomere maintenance by preventing telomere end-to-end fusion through proper folding of the telomere. The goal of this study was to describe the regulation of expression of these genes and proteins along with their relationship to telomere length in an animal model comparing different diets and a simulated occupational exposure. Male Sprague-Dawley rats were maintained on a regular (REG) or high fat (HF) diet for 24 wk. At wk 7 during diet maintenance, groups of rats from each strain were exposed by inhalation of stainless-steel welding fume (WF; 20 mg/m^3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air until wk12, at which time some animals were euthanized. A separate set of rats were allowed to recover from WF exposure until the end of the 24 wk period. At 12 and 24 wk, the effect on shelterin proteins and telomere length was examined in peripheral blood mononuclear cells (PBMCs) and homogenized liver tissue. Double- and single-stranded telomere DNA-binding proteins Trf1/Trf2 and Pot1, as well as telomeric DNA damage foci λH2AX and 53BP1, were influenced at 12 wk and 24 wk by both diet and exposure. A significant reduction in telomere length in PBMCs was observed in the WF+REG and Air+HF groups, which was further shortened in WF+HF group. However, an opposite telomere length trend was observed in livers obtained from the same groups at both 12 and 24 wk. ATM kinase phosphorylation and DNA damage activation was observed in the liver at 12 wk of WF exposure. Single-stranded binding protein Pot1, initially up-regulated at 12 wk in the WF+REG group, was later down-regulated in liver tissue at 24 wk along with the WF+HF group. In conclusion, our data suggest that disruption/down-regulation of single-stranded Pot1 and double-stranded Trf1/Trf2 expression in liver might act as an anti-apoptotic mechanism in the DNA-damage response leading to multistep liver injury by involvement in the telomere response to diet changes and WF exposure.

1246 A Comparative Analysis of KIM-1 as a Kidney Injury Biomarker in Rat: Plasma and Urine Protein Levels and Kidney Gene Expression

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Kidney injury molecule-1 (Kim-1), also known as T-cell immunoglobulin mucin-1 (TIM-1) and hepatitis A virus cellular receptor 1 (havrc1) is now a well-established urinary biomarker for detection of kidney proximal tubule injury in rat and human. It has been shown that urinary Kim-1 outperforms traditional serum biomarkers blood urea nitrogen (BUN) and serum creatinine (SCr). However, unlike blood, urine is often not collected in animal studies conducted early in drug development and therefore a sensitive blood-based biomarker of kidney injury would be beneficial. We have evaluated Kim-1 as a potential blood-based (plasma) kidney toxicity biomarker and compared its performance to that in kidney tissue and urine, and also to the performance of BUN, SCr and histopathology outcomes in 10 rat *in vivo* studies with known kidney toxicants. To confirm specificity, Kim-1 was also measured in plasma of 11 rat studies with target organ toxicities other than kidney. The most sensitive Kim-1 endpoint (highest fold change observed) was gene expression in kidney. RNA increases were occasionally detected prior to the onset of injury as defined by histopathological changes as shown by ROC exclusion and inclusion models (AUC exclusion = 0.99, AUC inclusion = 0.81). While urinary Kim-1 generally matched kidney RNA expression, smaller fold changes observed with RNA were often not observed when urinary Kim-1 was evaluated. Plasma Kim-1 was the least sensitive method of kidney injury detection, increasing only when higher grade kidney injury was observed, and its performance was comparable to that of the BUN and SCr biomarkers (AUC BUN 0.84, KIM-1 0.85, SCr 0.78 in exclusion model). Plasma Kim-1 is mostly unchanged in non-kidney injury studies; however, in cases where T-cell numbers are decreased (cyclophosphamide) or increased (concanavalin A), plasma Kim-1 reflects those changes, indicating that the source of plasma Kim-1 could be both immune cells and kidney. In conclusion, even though plasma Kim-1 increases with kidney injury, it did not outperform traditional serum biomarkers BUN and SCr. Kim-1 gene expression analysis followed closely by urinary Kim-1 protein measurement are the most sensitive Kim-1-based endpoints to detect kidney injury.

1247 Detection of Anti-Polyethylene Glycol IgM Antibodies in Healthy Individuals

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Polyethylene glycol (PEG) is a biocompatible polymer that is widely used in biopharmaceuticals to increase bioavailability, reduce frequency of administration, and optimize pharmacokinetics. Anti-PEG antibodies have been detected in healthy individuals and may result in decreased efficacy and alter pharmacokinetics of pegylated biopharmaceuticals; however, the prevalence of anti-PEG antibodies is unclear. We have developed a flow cytometry assay to detect anti-PEG IgM antibodies and have assessed their presence in the plasma of 300 heathy individuals. Anti-PEG IgM antibodies were observed in 45% of the individuals, with values ranging from 15 ng/ml to 12 µg/ml. Their presence was confirmed by Western blotting assay. The prevalence of positive samples was 60% in individuals 15-24 years old, 43% in individuals 25-64 years old, and 20% in individuals ≥ 65 years old. The prevalence of anti-PEG IgM antibodies did not differ between sexes or among races (Black, Caucasian, or Hispanic). Our study indicates that the flow cytometry can be used to measure the presence of anti-PEG IgM antibodies in healthy individuals.

1248 Can Ethyl Glucuronide in Hair Be Associated with Alcohol Use in Heavy Drinkers?

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Ethyl Glucuronide (EtG) in hair is proposed as a biomarker for assessment of long-term alcohol consumption. The present study evaluated the association between EtG in hair with alcohol use. Using cross-sectional study design, ninety-one alcohol dependent patients (diagnosed as per International Classification of Diseases, Version-10) with last alcohol consumption within 24 hours were recruited after their consent. The subjective information included: socio-demographic details, alcohol use details and alcohol amount consumed in past three months (by beverage-specific quantity-frequency method). Three centimetre of hair from the posterior vertex region of the head was collected and analysed using gas chromatography-mass spectrometry. The obtained EtG values were compared and correlated with the amount of alcohol consumed. The mean age of the participants was 37.7 (SD: 7.7) years. All participants used alcohol daily, locally brewed liquor being the preferred beverage (51.6%). The mean age of onset of daily alcohol consumption was 27.7 (SD:6.3) years and the mean age of onset of early morning drinking was 32.8 (SD:7.3) years. Mean quantity of alcohol consumed in past three months was 261.7 grams per person per day. All hair samples showed EtG value higher than the cut-off (i.e. 30pg/mg). EtG values expressed a positive correlation ($r = 0.508$, $p = 0.01$) with quantity of alcohol consumed. A simple linear regression was calculated to predict EtG values based on amount of alcohol consumed in last three months, $b= 0.608$, $f (89) =4.213$, $p<0.001$. A significant regression equation was found; $F (1,89) = 52.084$ $p<0.001$, with an R^2 of 0.369. Wilcoxon signed rank test showed statistically significant differences between the hair EtG and quantity of alcohol consumed ($Z= -8.28$, $p<0.001$). The study showed that EtG hair can also be objectively used to assess the quantity of alcohol consumed.

1249 Comparison of Routine Clinical Pathology Parameters of Sprague Dawley Rats by Different Blood Collection Sites

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The Sprague Dawley rat (*Rattus norvegicus*) is commonly used in preclinical toxicity studies. Blood collection sites from either abdominal aorta or vena cave at termination are routinely used for a preclinical study. However, it is certainly necessary to evaluate the drug potential toxicity at interim period of a given study by collecting blood from the jugular vein for clinical pathology evaluations for some sub-chronic and/or long-term toxicology studies.

Therefore, it is important to evaluate the variation of clinical pathology data by the different sites. A comparison of common hematology and clinical pathology data from different blood collection sites, i.e., at the abdominal aorta and the jugular vein, was made. Approximately 30 animals' data for each sex was collected. Data of control animals from completed toxicology studies were used to see if there were any difference for clinical pathology data at different blood collection sites. Analysis of the clinical chemistry data showed that significantly reduced glucose (GLU) (98% for male and 76% for female) was noted for the blood samples from jugular vein when compared with the samples from abdominal aorta. Slightly increase in total protein (TP), albumin (ALB), alkaline phosphatase (ALP) were observed for the samples from jugular vein compared with the samples from abdominal aorta. Difference in clinical hematology parameters included increases in leukocyte (WBC), erythrocyte (RBC), and hemoglobin (HGB) the samples from jugular vein relative to the samples from abdominal aorta. Analysis of clinical chemistry showed that a moderate difference was noted at glucose due to blood collection site difference. Whilst the data showed no significant difference in rest parameters of clinical pathology between the two sites. It is strongly recommend that the separate data sets are maintained for the interpretation of clinical pathology data.

PS 1250 A 12-Month Randomized Controlled Trial Evaluating the Effects of Switching from Smoking to Using a Tobacco Heating Product on Health Effect Indicators: Preliminary Findings

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Tobacco harm reduction; replacing smoking with potentially reduced-risk nicotine products, could offer substantial health benefits to those who will otherwise continue to smoke. Confined 5-day clinical studies have shown that toxicant exposure reduces when using the glo tobacco heating product compared to smoking cigarettes. While the short-term confinement data are encouraging, longer term data is needed to determine if toxicant reductions are sustained and if those reductions translate into an improvement in a variety of health effect indicators. This was investigated in the current study. Regular smokers were randomized to either continue smoking (CTS; N=79) or glo use (N=197) for one year. Arms of never-smokers (N=40) and of regular smokers intending to quit (N=190) who were provided with assistance to do so were also enrolled. Biomarkers of exposure (BoE) to a suite of cigarette smoke toxicants were measured at baseline and throughout the study. Given the potential for product use compliance issues in the study cohorts, the acrylonitrile haemoglobin adduct; *N*-(2-cyanoethyl)valine (CEVal) was also measured to provide longer-term information on non-compliant combustible product use. A planned interim analysis was performed on a subset of subjects (CTS (N=33), glo (N=76), cessation (N=133) and never-smoker (N=37)). Rapid and sustained decreases from baseline for BoE endpoints in the glo arm were observed, including markers for NNK (110 ng/24h; 60%), benzene (3.55 µg/24h; 89%), acrylonitrile (151 µg/24h; 91%), crotonaldehyde (313 µg/24h; 74%) and 1,3-butadiene (2.92 µg/24h; 86%), approaching or reaching similar levels to the smoking cessation and/or never-smoker arms. Furthermore, preliminary data for biomarkers of potential harm (BoPH) show improvement in the areas of oxidative stress, inflammation and platelet activation. Pre-specified thresholds for CEVal indicate a high level (80%+) of compliance in this interim analysis. The findings demonstrate that when smokers switched from smoking combustible cigarettes to using glo, reductions in their exposure to smoke toxicants were sustained for the 90-day period, and favourable changes were also observed in BoPH. This shows that glo is a potentially reduced exposure tobacco product.

PS 1251 Gulf War Illness as a Potential Autoimmune Disease: Neurodegeneration-Induced Autoantibodies against Neural Proteins

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Previously, we used our autoantibodies against neural proteins to develop diagnostic markers in veterans of the 1991 Gulf War (GW). Approximately one third of the American military personnel who served in the GW experienced chronic symptoms of Gulf War Illness (GWI), including: fatigue, headaches, memory and concentration problems, joint pain and gastrointestinal distress. In the present study, we used western blot assay to screen plasma for the presence of autoantibodies (AA) against the following neural proteins: neurofilament triplet proteins (NFP), tubulin, microtubule associated tau proteins (tau), microtubule associated protein-2 (MAP-2), myelin basic protein (MBP), myelin associated glycoprotein (MAG), calcium-calmodulin kinase II (CaM-KII) and glial S100B protein. Plasma reactivity was measured as arbitrary chemiluminescence units. The study included 180 veterans with GWI and 52 non-veteran asymptomatic controls. None of the participants showed any significant change in the level of AA against S100B. Veterans with GWI showed increased AA when compared with controls in descending order: MAP-2 > MBP > NFP > Tubulin > Tau > MAG > SNCL > GFAP > CaMKII. We have demonstrated that veterans with GWI had consistent patterns of increased autoantibodies against neural proteins. These autoantibodies may also be markers of disease and may contribute to the pathogenesis of GWI (i.e, they may be the cause for the symptoms). Such autoantibodies can cross the damaged BBB into the brain resulting in brain alterations characteristic of GWI. Our conclusion is consistent with results other recent studies. We hypothesize that neurodegeneration, resulting from combined chemical exposures in GW veterans, is the cause of GWI. Following brain injury neural proteins cross the breached BBB into circulation, triggering the formation of IgG autoantibodies. We propose that these circulating autoantibodies may serve as biomarkers for screening, diagnosis and treatment of GWI. *Supported in part by DOD Contract No. W81XWH-15-1-0641, W81XWH-15-1-0640, and W81XWH-18012.*

PS 1252 Impaired Toll-Like Receptor 3 (TLR-3) and Retinoic Acid Receptor Beta (RARβ) Expression in Poly I:C Activated Prostate Stem Cells Malignantly Transformed with Sodium Arsenite

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Cancer stem cells (CSCs) are associated with the initiation, progression and recurrence of cancer. The non-tumorigenic prostate epithelial cell line, RWPE-1, chronically exposed to iAs develops into an aggressive cancer cell line (CAsE-PE) and shows increased expression of CSC markers SOX2, Notch-1, CD44 and CD133, as well as multidrug resistance proteins. A therapeutic strategy in prostate cancer is the administration of agents that promote the differentiation of CSCs, such as retinoic acid (RA), which interacts with its specific receptor (RARβ) to induce the expression of genes associated with differentiation and decrease CSC markers. The co-administration of RA with the synthetic ligand of TLR3, polyinosinic:policitidylic acid (PIC), induces both inflammatory and pro-apoptotic responses, as well as the differentiation of CSCs by increasing the expression of RARβ. Synergy between RA and PIC has proven to be effective in prostate cancer *in vitro* models, sensitizing CSCs to conventional chemotherapies and inducing apoptosis. However, our group demonstrated that the iAs malignantly-transformed cell lines CAsE-PE and As-CSCs, show a significant decrease in TLR3 expression, suggesting that the combination of RA+PIC may be ineffective in inducing cell death in these cells. The aim of this study was to determine whether the administration of PIC+RA can induce apoptosis and cell differentiation in these iAs-transformed cell lines. As-CSCs and CAsE-PE, and their respective normal counterparts, WPE-stem and RWPE-1, were exposed to PIC, AR or the combination. Results showed that in As-CSC and CAsE-PE cells, the combination of RA+PIC did not modify the expression of SC markers CD44, CD133 and SOX2, nor induce significant apoptosis, compared to non-transformed parental cells, RWPE-1 and WPE-stem. These results suggest that iAs induces a decrease in TLR3 expression making these cells non-responsive to the treatment with PIC+RA.

PS 1253 Identification of Mechanistic microRNA Biomarkers for Ketoconazole-Induced Liver Injury

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Drug induced liver injury is a major reason for drug withdrawal from the market and termination of clinical trials during drug development. Increasing studies have indicated that microRNAs (miRNAs) are critical modules in hepatotoxicity. However, the potential of using miRNAs as robust and reliable biomarkers for hepatotoxicity remains to be further elucidated, especially via uncovering the underlying mechanisms. In this study, we employed a systematic approach to identify mechanistically-meaningful miRNAs associated with hepatotoxicity induced by ketoconazole. We performed miRNA-sequencing with liver tissues from Sprague-Dawley rats treated with ketoconazole at three doses (10, 30 and 100 mg/kg) and four time points (3, 7, 14 and 28 days).

We profiled miRNA expression using the miRDeep2 software and identified differentially expressed miRNAs (DEMs) at different treatment conditions using DESeq2, an R/Bioconductor package. The time- and dose-dependence of miRNA differential expression were then examined to explore miRNAs with early and sensitive response to ketoconazole treatment. To understand the mechanism underlying the association of the DEMs with ketoconazole-induced hepatotoxicity, we identified protein coding genes targeted by these DEMs via computation prediction. Using ArrayTrack and Ingenuity Pathway Analysis tools, we conducted pathway analysis to identify biological pathways that DEMs and their target genes were enriched in. Finally, candidate miRNAs responsible to ketoconazole-induced hepatotoxicity were proposed.

1254 Circulating miRNA Signatures Associated with NAFLD in Obese Adolescents

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Accumulating evidence indicates circulating microRNAs (miRNAs) potentially play important roles in metabolic organ crosstalk, thereby acting as non-invasive biomarkers or indicators for diagnosis and progression of metabolic disorders. In this study, we aimed to investigate serum miRNA signatures of obesity-associated non-alcoholic fatty liver disease (NAFLD) in adolescents and identify circulating miRNA markers to improve the risk prediction of NAFLD in obese children. Thirty-five, obese (BMI≥ 95th percentile), adolescents (aged 10-17 years) were recruited from a weight management clinic. Fasting blood sampling and liver MRI for hepatic fat quantification were performed at the initial visit and a 6-month follow up visit. Serum miRNA signatures were determined by quantitative RT-PCR-based miRNA array (180 miRNAs) and their associations with clinical indices, i.e. ALT, HOMA-IR, were analyzed using Pearson's correlation analysis. Profiled miRNAs were assessed using ROC curve analysis to discriminate NAFLD (hepatic fat ≥ 5%) versus non-NAFLD (hepatic fat < 5%). Seven miRNA expressions, miR-150, -221, -155, -122, -24a, -21, and -34a were significantly increased in the NAFLD group compared to the non-NAFLD group ($p < 0.05$). Of them, miR-34a and miR-122 were positively correlated with ALT levels ($p < 0.05$) and were potential discriminators between NAFLD and non-NAFLD (AUROC = 0.782, $p = 0.005$ for miR-34a; AUROC = 0.741, $p = 0.016$ for miR-122). Moreover, mir-21, which is associated with steatohepatitis, was highly expressed (5.6-fold) in the NAFLD group at the 6-month follow up compared to the initial visit ($p < 0.05$). These findings support the use of miRNAs as biomarkers for NAFLD-diagnosis and progression in adolescents.

1255 Optimization of Tissue and RNA Preparation to Facilitate RNA-Seq Analysis of Metabolic Syndrome Biomarkers in a Diversity Outbred Mouse Population

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With obesity steadily rising in the US, development of assay systems that enable identification of adverse chemical effects on a backdrop of genetic variability and metabolic disease becomes increasingly important toward protection of public health. The NTP is investigating use of the Diversity Outbred (DO) mouse population as a surrogate for human diversity and has performed a 13-week high fat diet study to investigate transcriptional biomarkers that inform development of metabolic disease in susceptible individuals. As part of a summer undergraduate internship at NIEHS, RNA isolation conditions were optimized from three tissues relevant to metabolic syndrome - skeletal muscle, liver, and white adipose. To optimize the experimental workflow, a pilot study was performed to optimize variables that affect RNA yield and integrity. Test variables included the tissue disruption tube type and the effect of freezing post-homogenate tissue samples. Tissues were homogenized in either Lysing Matrix A or Lysing Matrix D tubes (MP Biomedical). The D tube proved to be the most favorable choice for complete tissue disruption. Following tissue homogenization, RNA was extracted, and the yield and integrity assessed. RNA concentrations from 10 mg liver (68.5 ng/µL to 259 ng/µL) and 10 mg muscle (3.5 ng/µL to 57.1 ng/µL) were acceptable for RNA-seq analysis. Increasing tissue sample input from 10 mg to 60 mg yielded satisfactory concentrations for adipose (11 ng/µL to 69 ng/µL). There was no significant difference in RNA quantity or quality between fresh and frozen post-homogenized samples (RNA Integrity Number greater than 8). However, for efficient workflow optimization, freezing the homogenates increased sample throughput. In summary, there was no significant difference in RNA yield or quality when freezing the tissue homogenate, therefore frozen homogenate was used to optimize the workflow. Lysing matrix tube D was preferable to use because there was less foaming, complete tissue disruption, and a clear homogenate. 10 mg of liver tissue, 20 mg of muscle tissue, and 60 mg of adipose tissue was used for RNA extraction. Optimized assay conditions identified here were carried forward to the DO transcriptomic study.

1256 Exploration of Small RNA Biomarkers in Serum Exosomes for Testicular Injury in Rats

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Testicular injury is often observed in a drug development. Serum hormones are usually used as non-invasive biomarkers for testicular injury. However, sensitivities of the markers are low. Therefore, it is difficult to monitor testicular injury in pre-clinical and clinical drug developments. In recent years, molecules in body fluid exosomes attract attention as disease biomarkers such as cancer. In this study, small RNAs in serum exosomes were analyzed for identifying non-invasive biomarkers of testicular injury in rats, which are mainly used in a pre-clinical drug development. Testicular injury models in rats were prepared by a single oral administration of 2000 mg/kg ethylene glycol monomethyl ether (EGME) or 400 mg/kg carbendazim (CBZ). Spermatocytes degeneration and sertoli cells vacuolation were observed in the testes in EGME-administered rats. Spermatocytes degeneration and dilation of seminiferous tubules with shedding of seminiferous epithelial cells were observed in CBZ-administered rats. Exosomes were isolated from serum of these models by an ultracentrifugation method, and exosomal small RNA-seq analysis was performed. The analysis identified some small RNAs that fluctuated in common between the two models, and selected miR-423-5p and miR-128-3p as candidate markers. For qPCR validation of the candidate markers, testicular injury models were further prepared by a single oral administration of 60 mg/kg 1,3-dinitrobenzene (1,3-DNB) or 500 mg/kg nitrofurazone (NF) in which spermatocytes degeneration and sertoli cells vacuolation were observed. In qPCR analysis, both exosomal miR-423-5p and miR-128-3p levels were upregulated in EGME, CBZ and NF models, but not 1,3-DNB model in which severe hemolysis was observed. On the other hand, the miRNA levels in serum samples did not change significantly in these models. In conclusion, we identified miR-423-5p and miR-128-3p in serum exosome as biomarkers for testicular injury in rats.

1257 Role of Iron Status in Manganese Toxicokinetics in Humans

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Manganese (Mn) is both an essential trace element and a toxic metal that shares similar properties with iron (Fe). Animal studies have shown that Mn is mainly excreted into feces and urine, and that increased absorption and retention of Mn could occur as result of iron-deficiency (ID). However, human evidence on Mn toxicokinetics is limited. This study is to examine the hypothesis that ID will promote the body retention of Mn due to decreased elimination in humans. The elimination of Mn was assessed by creatinine-adjusted urine Mn levels in the absence of fecal data. The analysis was restricted to 580 non-pregnant females aged 16-49 years who had valid urinary and blood Mn and Fe measurements as part of the 2015-2016 National Health and Nutrition Examination Survey. Iron status was assessed by total body iron (TBI) scores that was calculated from measured serum ferritin and transferrin receptor. Iron deficiency was defined as TBI score <0. Demographic factors including age, race, body mass index (BMI), smoking, socioeconomic status, and glomerular filtration rate were examined in the multiple linear regression analysis. Logarithmic transformations were applied when necessary. In this current analysis, an estimated 8.5% (age-standardized) of the study population has ID. Whereas there is a positive correlation between blood and urine Mn levels (Spearman correlation= 0.08, p =0.09), the unadjusted analysis showed that the ratio of urine-to-blood Mn was significantly lower in subjects with ID than their counterparts (0.15 vs. 0.19 dL/g creatinine). Similar findings were observed that the ratio of interest was approximately 30% lower in individuals with ID compared to those without ID in the multivariate analysis (p =0.02). Of the covariates examined, race and BMI were the predominant determinants that the urine-to-blood Mn ratio tended to be lower in African Americans and those with higher BMI. In summary, this analysis suggested that iron status may play a role in Mn toxicokinetics. Further studies in humans that include urinary and fecal measurements are warranted to confirm the current findings. A better understanding of the key determinants in the body burden of Mn, one could further advance Mn exposure assessment.

1258 Circulating Cell-Free DNA Concentrations in CD-1 Mice following a Combination Exposure of the Antiretroviral Drugs Emtricitabine, Tenofovir, and Efavirenz

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There is keen interest in the clinical and toxicology disciplines to explore circulating cell-free DNA (ccfDNA) as a blood-based biomarker of diagnostic and predictive value. Current blood-based applications for ccfDNA in the clinical realm include oncology, fetal diagnostics, organ transplantation, and autoimmune disorders. The aim of this study was to evaluate changes in levels of ccfDNA as a toxicological indicator in a rodent model following *in utero* exposure to a standard combination antiretroviral therapy (ART). Plasma ccfDNA was quantified in time-mated CD-1 mice administered vehicle control (0.5% methylcellulose in water) or a drug cocktail [Emtricitabine (50 mg/kg), Tenofovir (75 mg/kg), and Efavirenz (150 mg/kg)], non-treated pregnant, and non-pregnant vehicle controls. Pregnant dams (F0) were dosed by oral gavage starting on GD 5 (gestational day 5) through PND 20 (postnatal day 20). First generation (F1) male and female pups were continuously dosed from PND 13 until the day prior to euthanasia (PND 35). Whole blood was collected on GD 15 (F0), PND 21 (F0), and PND 35 (F1). ccfDNA was extracted from plasma, quantified, and sample integrity determined by DNA fragment analysis. In GD 15 dams, ccfDNA concentrations of the pregnant vehicle control, treated and non-treated dams were significantly elevated (27% increase) compared to the non-pregnant vehicle control mice, indicating pregnancy itself accounted for increased ccfDNA. This result agreed with human ccfDNA findings in pregnant women. By PND 21, ccfDNA levels in non-treated dams returned to normal, baseline non-pregnant control values. Even though increased levels were observed at PND 21 in vehicle control and treated dams, the difference was not statistically significant. ART exposure in pregnant and lactating mice, and their offspring (PND 13 through 35) did not affect ccfDNA concentrations. The ccfDNA data correlated with an absence of a body weight, food consumption, or clinical observation study phenotype. Despite the lack of chemical-mediated effects on ccfDNA levels, this study demonstrated that liquid biopsy by ccfDNA measurement may be a viable tool to screen responses to xenobiotic agents in rodent toxicology studies.

1259 Upregulation of Metastasis-Associated Lung Adenocarcinoma Transcript 1 and Growth Arrest Specific 5 Genes in Prostate Cancer Subjects in Southern Nigeria: A Risk for Genome Protection

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Prostate cancer is the most diagnosed solid tumour and second leading cause of cancer related deaths worldwide. A number of toxic metals have been reported to have direct genotoxic effect and disruption of gene repair mechanisms in prostate carcinogenesis. There is currently a dearth of information in the status of genotoxicity biomarkers and the use of prostate specific genes in the diagnosis of prostate cancer in Nigeria. This study is aimed to evaluate the expression of some prostate specific genes [Metastasis-associated Lung Adenocarcinoma Transcript 1 (MALAT-1) and Growth Arrest Specific 5 (GAS 5)], antioxidant enzymes: superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GRx), catalase (CAT) and the status of genotoxicity biomarkers: wild type tumour suppressor protein 53 (p53), DNA repair protein: 8-oxoguanine DNA glycosylase (OGG_1) and oxidative DNA damage biomarker: 8-hydroxydeoxyguanosine (8-OHdG) and correlate with toxic and essential metals in 150 prostate cancer subjects, 100 benign prostatic hyperplasia subjects and 100 age-matched control subjects in Southern Nigeria. From the blood samples collected, Reverse transcriptase polymerase chain reaction (RT-PCR) method was used to determine expression of prostate specific genes, Spectrophotometric methods to determine antioxidant enzymes in serum, Enzyme linked immunosorbent Assay was used to determine the genotoxicity biomarkers in serum while Inductively Coupled Plasma Mass Spectrophotometer (ICPMS) was used to determine toxic and essential metals levels in whole blood. From the results, we observed upregulation of MALAT-1, GAS 5 genes, significantly higher levels of toxic metals and 8-OHdG, significantly lower levels of essential metals and antioxidant enzymes in prostate cancer subjects. Additionally, there was down regulation of p53 and OGG_1 in prostate cancer subjects compared to benign prostatic hyperplasia and control subjects. There data provides evidence of upregulation of prostate specific genes, oxidative stress and DNA damage as reflected in heavy toxic metal burden, lowered antioxidants defense system, down-regulation of p53 and OGG_1 which is a key. These finds are suggestive of possible disruption of the genome protection mechanism, a key mechanism in the development and progression of prostate cancer.

1260 Chronic Electronic Cigarette Use Elicits Changes in Biomarkers Related to Pulmonary Pathogenesis

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Electronic cigarettes (e-cigs) primarily originated as smoking cessation devices aimed at modifying the risk of developing pulmonary diseases associated with combustible tobacco use. However, more frequently never-smokers are adopting e-cig use *de novo*, and the relative safety of chronic exposure to e-cig vapor remains unclear in terms of lung pathogenesis. Thus, this study aims to evaluate gene/protein biomarkers, which are associated with cigarette-induced chronic obstructive pulmonary disease (COPD) and/or idiopathic pulmonary fibrosis (IPF), in animals chronically exposed to nicotine containing e-cig vapor. C57BL/6J mice (n=15/group) were randomly assigned to one of three 8-month exposure groups: e-cig vapor (commercially available, 18 mg/mL nicotine), UK 3R4F reference cigarette smoke, or filtered air as a control. Lung tissues and paraffin embedded slides were used to evaluate gene and/or protein expressions of COPD and IPF biomarkers from the CYP450 metabolism (CYP2A5, CYP3A11), oxidative stress (superoxide dismutase 1 (SOD1)), epithelial–mesenchymal transition (E-cadherin, vimentin), and survival/apoptotic pathways (p-AKT, B-cell lymphoma extra-large (BCL-XL), p53, p21, and chromosome region maintenance 1 (CRM1)). Results from the cigarette group were consistent with previously published studies. Expressions of E-cadherin and CRM1 were significantly decreased in the e-cig group as compared to the control group (E-Cadherin: <75.3%, CRM1: <74.5% of control; $p<0.05$). Expressions of SOD1 and BCL-XL were significantly upregulated in the e-cig group as compared to the control group (SOD1: 135%, BCL-XL: >151% of control; $p<0.05$). Nuclear sub-cellular localization of p53, evaluated by immunohistochemistry staining, in bronchiolar tissues was higher in the e-cig group (25.3±0.9%) as compared to controls (12.1±0.6 %) ($p<0.01$). Moreover, there were few significant differences observed between the e-cig and cigarette groups. As these related molecular changes are involved in the pathogenesis of cigarette-induced COPD and IPF, the possibility exists that e-cigs can produce a similar outcome, although further investigation is warranted.

1261 Cell-Free Circulating microRNAs Associated with Arsenic Exposure through Drinking Water Reflect Environmental Health Risks

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Arsenic (As) is a toxic metalloid which is naturally present in the environment throughout the air, water and land. The greatest public health threat of As arises from high levels in the groundwater which result in contamination of drinking water, food and crops. The WHO estimated that worldwide more than 200 million people are chronically exposed to As levels that are higher than the prescribed limits. Epidemiological studies have shown that long-term exposure to As increases the risk of developing cancer as well as cardiovascular, metabolic, and more recently also multiple liver diseases. Recently, cell free, circulating microRNAs (cimiRNAs) have shown a promise as biomarkers of environmental exposure and health effects. In this study we explored the potential of cimiRNA signatures for biomarker based risk-assessment of chronic As exposure. Here, we present a global analysis of cell-free, cimiRNAs in a human population (n = 24) upon As exposure through drinking water in a rural setting of Punjab in southeast Pakistan. We determined urinary As levels using atomic absorption spectrophotometer coupled with hydride generation as a proxy for the internal As exposure and analyzed global plasma cimiRNAs using next-generation sequencing technology across all subjects. Lastly, we applied linear mixed effect models to identify associations between internal As levels and cimiRNA levels, thereby adjusting for batch effects, sex, age, BMI as well as genotype (False discovery rate < 0.2). The data revealed a signature comprising of 14 cimiRNAs to be significantly associated with internal As levels. Interestingly, the cimiRNA signature featured multiple emerging biomarkers for various types of liver injury (miR-122-5p and miR-192-5p), type 2 diabetes mellitus (miR-375-5p and miR-181b) and colorectal cancer (miR-17-3p), as well as the anti-inflammatory miR-146a-5p. Despite the limited

sample size, the present study provides molecular insights into the health effects of chronic As exposure. Although more confirmatory studies are needed, this study indicates that the cimiRNA signature approach has great potential to improve biomarker based environmental health-risk assessment.

PS 1262 Urinary microRNA Profiles in Rat Models of Chronic Kidney Injury: Is there a Benefit of Exosome Enrichment?

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Exosomes are extracellular vesicles (EVs) secreted into various biofluids including urine. They enclose micro-RNAs (miRNAs), which are involved in the posttranscriptional regulation of gene expression. In the context of chronic kidney injury (CKI) like diabetic nephropathy (DN), altered exosomal composition and urinary miRNA profiles were reported previously. As exosomes are remarkably stable, they are considered as a promising source for miRNA investigations. We compared miRNA profiles of whole urine and urinary exosomes in two rat models of genetically and toxin-induced CKI: (1) Obese ZSF1 rats (14 to 26 weeks of age) and (2) Wistar rats with nephrotoxic serum-induced glomerulonephritis (GN). Animals were compared to respective controls. Kidney injury was confirmed in both models by histopathology and by quantification of the urinary proteins KIM1, OPN, CLU and CysC. EVs including exosomes were enriched using the miRCURY exosome precipitation kit (Qiagen). miRNAs from EVs and total urine were isolated by the miRNeasy kit (Qiagen) and quantified by miRCURY LNA qRT-PCR (Qiagen). Disease association and potential target genes were determined with Ingenuity pathway analysis (IPA) Qiagen). Exosomal association was assessed by qEV size exclusion chromatography (SEC) columns (Izon). Results were analyzed using the modified dCt method (Pavkovic et al 2014). Compared to ZSF lean rats, 67 EV-associated miRNAs and 52 miRNAs in total urine were significantly altered in ZSF1 obese rats from 14 to 26 weeks of age, with 8 miRNAs in common. IPA-derived annotation showed strong association with renal inflammation and glomerular injury, which is more prominent after EV-enrichment. In addition, many miRNAs have been described in the context of human DN, like members of the let-7 family, which we detected mainly in whole urine of obese ZSF1 rats. EV-enrichment added further miRNAs with implications in human CKI, some being described in the context of podocyte function. The exosomal association and significantly increased levels in urine of obese compared to lean rats were confirmed for selected miRNAs including miR-10a-5p by SEC. Increased levels of selected miRNAs were also found in urine of GN rats, supporting a role of miRNA in both subacute and CKI models. In summary these results show the added value of EV enrichment on miRNA detection, support a translational value of the ZSF1 animal model and contribute to further characterization of miRNAs in CKI.

PS 1263 Potentially Translatable Biomarkers for Monitoring Progressive Colorectal Cancer

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Colorectal cancer (CRC) is the third most common cancer among men and the second most common cancer among women worldwide. In Vietnam, CRC is the fifth most common cause of cancer-related incidence, with most patients being diagnosed at stage II. Several factors impact CRC treatment, including but not limited to tumor size, location, stage, phenotype (benign or malignant), local and/or metastatic recurrence, wellness of the patient, and modulation of signaling pathways [e.g. IGFBP7 (insulin-like growth factor binding protein 7)]. IGFBP7 is normally expressed in colonic mucosa and inactivated by DNA methylation in human CRC. Metabolomic studies revealed methylation of the tumor suppressor gene adenomatous polyposis coli (APC) and IGFBP7 gene promoter region in cancerous tissue. In combination with the predominance of methylation in normal tissue, this may serve as a prognostic indicator in CRC patients. Interestingly, Vietnamese CRC patients exhibit higher IGFBP7 gene methylation in colorectal adenoma versus matched normal tissue. Therefore, investigating molecular response profiles downstream IGFBP7 methylation could reveal novel biomarkers in CRC which is poorly predicted based on a single gene/protein. We explored the feasibility and potential usefulness of cytokine/chemokine profiling of tumors and adjacent normal tissues (as self-controls) in a cohort of 13 Vietnamese patients diagnosed with adenocarcinoma at different stages. Colorectal tissue levels of 41 different inflammatory mediators were measured using the MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel and Luminex MAGPIX platform. There were 22 out of 41 measurable cytokines/chemokines in normal and tumor tissues. Statistically significant increases in EGF ($p<0.0001$), TGF-α ($p<0.0001$), G-CSF ($p=0.0003$), Fractalkine ($p=0.0001$), INF-α2 ($p<0.0001$), GRO ($p=0.0178$), PDGFAB/BB ($p=0.0027$), IL-10 ($p=0.0069$), IL-12 ($p=0.001$), IL-12β ($p=0.0024$), IL-13 ($p=0.006$), IL-15 ($p=0.0163$) and MCP-1 ($p=0.0375$) were associated with increased severity of CRC. The tumor tissue-based changes in inflammatory mediators clustered with stage II adenocarcinoma at different severity grades, suggesting potentially translatable biomarker profiles may be monitored in tissue and/or liquid biopsy format(s) from animal models for CRC that exhibit normal or aberrant IGFBP7 methylation phenotypes during target validation and derisking studies as well as human clinical trials.

PS 1264 Cancer Driver Mutations as Quantitative Biomarkers of Cancer Risk: Interspecies Analyses Using CarcSeq

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Unmet needs in cancer risk assessment include the ability to predict rodent life-time tumor responses from short-term exposures and a scientific basis for rodent to human extrapolation. As an approach to address these needs, the use of hotspot cancer driver mutations (CDMs) as biomarkers of cancer risk was integrated with an error-corrected, next generation sequencing (NGS) approach. We developed a high-throughput NGS method called CarcSeq. The method involves performing multiple, high-fidelity PCR reactions to amplify the most prevalent mutations reported to occur in human tumors, tagging the amplicons with 9 bp unique identifier sequences at each end, constructing libraries using Illumina ChIP Seq kits, sequencing the libraries on an Illumina NextSeq500 platform and generating single-strand consensus sequences (SSCS), bioinformatically, as a means of error-correction. We validated CarcSeq mutant fraction measurements through observations related to known cancer driver tissue-specificities and mutation spectra for normal human breast and lung, ductal carcinoma, and lung adenocarcinoma samples. A reconstruction experiment established CarcSeq has a sensitivity of 10^4. We adapted this approach to analyze analogous conserved codons in rat and mouse. For each of the three species, the developed panel includes 13-15 amplicons encompassing known cancer driver genes (e.g., *KRAS*, *PIK3CA*, and *TP53*) encompasses the equivalent of 30 known human hotspot codons and has a combined target sequence length of ~1 kb. Amplicons were generated from DNA isolated from 16-week-old, untreated rodent tissue samples with known differences in spontaneous tumor incidence: female Wistar, Sprague Dawley, and F344 rat mammary samples and male and female $B6C3F_1$ and CD-1 mouse lung samples. The rat panel was optimized to generate ~100,000 SSCS/amplicon. CDMs were observed at conserved codons in young, untreated rats, and preliminary results are consistent with what was expected based on strain differences in spontaneous tumorigenesis.

PS 1265 Evaluation of Extracellular Vesicles (EVs) as Toxic Biomarkers in Mouse

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Recent findings reveal that extracellular vesicles (EVs), secreted from cells, are circulating in the blood. EVs are classified into exosomes (40-120 nm), microvesicles (50-1,000 nm) and apoptotic bodies (500-2,000 nm). EVs contain mRNAs, microRNAs, and DNAs and have the ability to transfer them from cell to cell. Recently, especially in humans, the diagnostic accuracy of tumor cell type-specific EVs as biomarkers is more than 90%. In addition, microRNAs contained in the EVs are being identified as specific biomarkers in blood for chemical-induced inflammation and organ damage. Therefore, microRNAs contained in the EVs released into the blood from tissues and organs in response to adverse events such as chemical substances are expected to be useful as novel biomarkers for toxicity assessment. In this study, we aimed to identify target organs by comprehensive analysis of EV RNAs in the blood of mice after chemical exposure, and establish a highly sensitive "Next Generation type" toxicity test. At first, we compared the protocols for the isolation of EV RNA from mouse blood, including blood collection methods, serum isolation methods, and exosome purification methods, to standardize the isolation of EV RNA from mouse blood. Quantity of exosomes were determined by Nanosight, a special optical microscopy adapted to quantify small particles, and western blot using anti-CD9 antibody. EV RNAs were further evaluated by deep sequencing. By using our optimized protocol, we succeeded in isolating more than 50 novel small RNAs, which could be used as novel highly sensitive biomarkers for hepatotoxicity due to carbon tetrachloride (CCl4). These results will accelerate a rapid evaluation of chemical substances and medicine in Nonclinical Safety Evaluation.

1266 Understanding the Context of Tobacco Smoke Exposure in a Cohort of Canadian Infants Using Biomarkers, Questionnaires, and Machine Learning

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Accurately assessing tobacco smoke exposure in early life is important to understanding and preventing childhood asthma. This study of the CHILD cohort study examined urinary concentrations of common nicotine biomarkers (cotinine and trans-3′-hydroxycotinine (3HC)) and evaluated their ability to be validated by questionnaire responses related to smoking. The low level of detection (0.03ng/mL), young study population, and low prevalence of reported smoking in this cohort add valuable knowledge to this area of exposure assessment. Urine was collected at 3-4 months of age during home visits. The concentrations were log-transformed and corrected for specific gravity. Multivariable linear regression (MLR) and random forest (RF) methods were used to assess the ability and relative importance of questionnaire responses to predict urinary cotinine concentrations. While just 12% of our sample (n=2509) reported recent tobacco smoke exposure to their child, 76% and 89% of the infants had detectable cotinine and 3HC levels. Questionnaire-based models incompletely predicted urinary concentrations, explaining only 32% and 41.0% of the variance in cotinine, and 3HC levels, respectively. Models may not adequately explain this variation because the half-life of cotinine leads to more variability for those with low or inconsistent exposure, questionnaires cannot adequately detect thirdhand smoke exposure, and/or detectable levels may in-part result from dietary nicotine sources. Breastfeeding, housing, and third-hand smoke exposures such as carpeting, appear to be important in explaining cotinine and 3HC concentrations, identifying priority areas for targeted disease prevention. Researchers must use context-specific exposure assessment measures, and the ability of machine learning to support purposeful analysis should be explored further.

1267 Identification of Metabolic Changes That Indicate Gentamicin-Induced Kidney Damage in the Laboratory Rat

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Identifying early indicators of toxicant-induced organ damage is critical to provide effective treatment. To discover such indicators and the underlying mechanisms of toxicity, we used gentamicin as an exemplar kidney toxicant and performed systematic high-throughput perturbation studies in Sprague Dawley rats and identified global changes in genes in the liver and kidneys, metabolites in the plasma and urine, and absolute fluxes in central carbon metabolism. We then used the measured changes in genes in the liver and kidney as constraints to a rat multi-tissue genome-scale metabolic network model to investigate the mechanism of gentamicin-induced kidney toxicity and identify metabolites associated with changes in tissue gene expression. Our analyses revealed that, compared to blood metabolites, urine metabolites have greater potential to serve as kidney-injury indicators, as several metabolites in the amino acid-, carbohydrate-, and lipid-metabolism pathways increased significantly as early as 7 h after gentamicin exposure. Thus, our analyses identified 1) several significantly enriched injury-specific pathways in the kidney underlying gentamicin-induced toxicity and 2) metabolites in these pathways to further target and clinically assess for their ability to serve as early markers of kidney damage. Together with our recent work on acetaminophen-induced liver toxicity, these results demonstrate how our platform can be broadly applied to analyze and mechanistically interpret large-scale high-throughput data to identify early indicators of organ injuries.

1268 Characterization of Linear and Non-Linear Associations between Physiological Indicators and All-Cause Mortality

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Numerous epidemiological and *in vivo* studies have documented associations between toxicant exposure and altered physiological indicators, but it is unclear how such chemical-mediated effects increase the risk of death. Characterization of the type of association (e.g., linear dose-response or non-linear) between physiological indicators and mortality will provide the foundation to understand how chemical exposures result in physiologic dysfunction associated with increased risk for death. In this study, we applied a machine learning approach to characterize the linear and non-linear associations between physiological indicators and mortality to identify populations who are at risk to physiologic dysfunction. We studied 27 biomarkers and physiological measurements in 47,025 participants of the US CDC National Health and Nutrition Examination Survey (NHANES) sampled from the US population from 1999-2014. We selected these particular indicators as they characterize physiological strain from a wide range of regulatory systems, have high sample size (>10,000 participants), and high overlap with the mortality data (>12 years). We used linked National Death Index to ascertain mortality status and time to death. We assessed linear associations with death and non-linear associations by discretizing each indicator into 9 quantiles. We conducted 10-fold cross validation (while adjusting for age, sex, and race/ethnicity) to compare the predictive capability of the linear versus non-linear models. Linearity was the worst at describing the associations between mortality and biomarkers of inflammation such as C-reactive proteins, white blood cell counts, and alkaline phosphatase with R2's of 0.06, 0.04, and 0.001, respectively and non-linearity improved the fit of the model by 3, 5, and 150 folds, respectively. For HDL, LDL, and a biomarker of renal function Glomerular Filtration Rate, we found that higher risk of death is associated with being in either extremes of the distributions instead of being in just one extreme. We present a framework to systematically characterize the type of association that best explains the relationship between a given physiological indicator and mortality. To better characterize toxicity associated with chemical exposures, understanding the associations between physiological indicators and mortality is salient to evaluate how toxicant exposure disrupts physiologic function that leads to higher risk of death.

1269 Demonstrability of an Enzyme-Linked Immunosorbent Assay to Simultaneously Quantify Histamine in Multiple Nonclinical Species and Matrices in a Single Analytical Run

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In investigational new drug enabling studies two species (rodents and large animals) are commonly tested. However, in most instances separate sample analytical procedures (AP) are developed for each species and matrix. The objective of this study was to validate an enzyme-linked immunosorbent assay (ELISA) to measure histamine from different matrices and species simultaneously in a single analytical run. Large quantities of histamine are released during an allergic reaction, but the timing of histamine release is critical, and hence multiple sampling is needed. Developing blood matrix-based assays where multiple sampling can be limited due to constraints in sample volume may be inadequate. Hence, it is more practical to develop an AP that can also measure histamine levels from urine where daily sampling is achievable. A commercially available histamine ELISA kit that cross reacts with NHP, canine, and rat histamine was used. A standard curve consisting of eight non-zero standards and quality control samples was established in assay buffer. Parameters such as blank checking to estimate the endogenous histamine concentrations, the determination of the optimal dilutions for study samples, and the evaluation of the expected histamine recovery in each matrix were conducted. Recovery was calculated by spiking with a known amount of histamine at three different concentrations into individual lots of matrices. Lastly, inter- and intra-assay precision, linearity, and endogenous quality control ranges were evaluated. A standard curve capable of determining histamine concentration from 0.098 to 25 ng/mL with acceptable accuracy and precision of 20% to 25% was established. The optimal dilution for sample analysis of NHP and canine plasma and serum was 1:10 and for urine it was 1:5. For rat plasma and serum, the dilution was 1:40 and for urine it was 1:20. The recovery (close to 100%) was established when samples were spiked with histamine at 0.188 ng/mL in all three species, except in canine serum, where it was 1 ng/mL. Inter- and intra-assay precision analyses in serum, plasma, and urine were established. In conclusion, we demonstrated the use of an AP in a

single run to simultaneously quantify histamine in multiple species and matrices. This experimental approach improves efficiencies and gives the possibility for repeated histamine sample analysis using urine samples.

1270 Altered SIRT1 Activity and Plasma Cortisol as Biomarkers of Circadian Disruption in Night Shift Workers

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Disruption of circadian rhythm by persistent night-shift showed to increase the risk of breast cancer. Identification and validation of biomarkers is essential to the development of preventive strategies for individuals, especially nurses, at elevated risk of breast cancer due to shift work. Our animal studies indicate that NAD^+/NADH and SIRT1 are key regulators of circadian gene expression, which play important roles in mammary carcinogenesis associated with disruption of circadian rhythm. We used a two-phase approach to assess NAD^+/NADH and SIRT1 activity in peripheral blood mononuclear cells (PBMC) as biomarkers for circadian disruption in night-shift workers. In Phase I, we analyzed circadian rhythm of NAD^+/NADH and SIRT1 activity in day- vs night-shift workers over 24 hr; in Phase II, we assessed the same markers in another group of day- vs night-shift workers at two selected time points. In the both phases, peripheral blood was collected and PBMC were isolated from plasma. NAD^+/NADH and SIRT1 activity were analyzed in PBMC, and melatonin and cortisol were analyzed in plasma. In Phase I (n=22), SIRT1 activity differed significantly over time points across a day with an increase from early to late night in day-shift workers, while the levels were largely unchanged over time in night-shift workers. These resulted in the largest difference in SIRT1 activity at late night (midnight) between day- and night-shift workers, although no statistical significance observed. NAD^+/NADH did not have any significant differences between day- and night-shift workers, and between early and late night. In Phase II (n=39), a similar pattern was observed with a late night increase in SIRT1 activity in the day-shift but not in the night-shift workers, showing a low to moderate reliability. In addition, while the plasma melatonin did not show difference over 24 hr in day- vs night-shift workers with a peak at 4 am, night-shift dampened rhythmic secretion of cortisol over a day, resulting in significant reduction of cortisol level at morning and further misalignment of cortisol to melatonin. These preliminary results suggest that suppression of the SIRT1 activity increase at late vs early night, and suppression of early morning plasma cortisol warrant further investigation as biomarkers of circadian disruption in night-shift workers. *Supported by 1 NIH/NIEHSR21 and 1 NIH/NIEHS ES005022-27.*

1271 Proposed Biomarkers from Lipidomics Analysis for Ethionamide-Induced Hepatic Steatosis in Rats

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Drug-induced hepatic steatosis is one of the adverse liver events often encountered in clinical trials or non-clinical toxicity studies and is recognized to be, in some cases, a serious concern in the development of new drugs. Therefore, a biomarker for drug-induced hepatic steatosis could be an important tool for managing the risk for new drug development. Ethionamide (ETH), a second-line drug for multi-drug resistant tuberculosis, is known to cause hepatic steatosis followed by hepatic injury in rats and humans. To find a suitable biomarker for ETH-induced steatosis, we performed lipidomics analysis using plasma and liver samples from steatosis model rats treated orally with ETH at 30 and 100 mg/kg for 7 and 14 days. The ETH-treated rats showed increased hepatic content and decreased plasma levels for triglycerides and total cholesterol on day 14 with 100 mg/kg, and they developed hepatic steatosis, shown by Oil Red O staining-positive vacuolation in the centrilobular hepatocytes on day 14 with 30 mg/kg and on days 7 and 14 with 100 mg/kg. A multivariate analysis for lipid profiles in the liver and plasma obtained on day 14 revealed that there were differences in the 35 lipid species in the plasma and 35 lipid species in the liver between the control and the ETH-treated rats at 100 mg/kg. Of those lipids, phosphatidylcholine (PC[18:0/20:4]) and lysophosphatidylcholine (LPC[18:0]) decreased dosedependently in both plasma and liver after administration of ETH. Since arachidonic acid (20:4) containing PC is known to be related to the secretion of very low-density lipoproteins from the liver, the decrease in PC[18:0/20:4] is considered to be involved in ETH-induced hepatic steatosis. In conclusion, plasma PC[18:0/20:4] and LPC[18:0] were considered to be appropriate biomarkers for ETH-induced hepatic steatosis.

1272 Next-Generation DILI Biomarkers: Prioritization for Qualification and Best Practices for Biospecimens

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Drug-induced liver injury (DILI) remains a diagnostic and clinical challenge in drug development. The qualification of emerging biomarkers capable of predicting DILI soon after the start of treatment, differentiating DILI from underlying disease, identifying the causal entity, and assigning appropriate treatment options after DILI is diagnosed are needed. Qualification efforts have been hindered by lack of properly stored and consented biospecimens that are linked to clinical data relevant to a specific context of use. Recommendations are highlighted for biospecimen collection procedures and qualification efforts should be focused by using a few specific emerging biomarkers, such as GLDH, HMGB1, K18, MCSFR1, OPN, and bile acids. These are based on extensive literature review and consensus achieved between the International Consortium for Innovation & Quality (IQ) DILI members and academic and regulatory experts. Member companies (11) of IQ DILI responded to a survey to provide insight into how samples are collected and stored, and on the availability of samples and data that can be used for qualification. Key takeaways include (i) Collect, with broad and ethical consent, and properly store biospecimens in clinical trials following procedures that allow for qualification of biomarkers. (ii) Collect clinical data concurrent with biospecimen and store data in a manner that is easily linked to the biospecimen, using graphical tools if possible. (iii) If DILI risk is predicted, collect data on emerging biomarkers, either during clinical trial development or a *post hoc* study setting, to advance biomarker qualification efforts. Additional clinical research is needed to develop DILI biomarkers. There are a few that are currently considered to be exploratory by the Food and Drug Administration and the European Medicines Agency. With the unmet need, there is opportunity to collaborate and advance the understanding of the diagnosis, management, and prevention of DILI by driving forward the qualification of biomarkers. *Acknowledgements: Mark I. Avigan, MD CM, US FDA.*

1273 Stratum Corneum Biomarkers of Skin Barrier and Immune Response in Patch Test Reactions to Common Contact Allergens and Irritants

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Patch testing is a commonly used diagnostic tool for contact sensitization (CS), however it is associated with false-positive and false-negative outcomes. The patch test baseline series contains several hapten mixes in one patch test preparation. In case of a positive reaction to a mix, patch testing of the individual haptens is performed to identify the causative hapten(s). However, some subjects show a negative reaction in subsequent testing which might indicate a previous false-positive, possibly irritant reaction. As irritant and allergic reaction have different pathophysiology (CS is a type IV hypersensitivity reaction involving T-cells while activated innate immunity and skin barrier damage underlie skin irritation), we investigated whether suitable biomarkers might assist in discrimination between irritant and allergic reaction. In the present study, we determined various biomarkers of skin barrier and immune response in patch test to fragrances. We included 23 persons with previously positive patch test to at least one compound from the baseline fragrance mix I and II. These subjects underwent subsequent patch testing with the single fragrances. All allergens were applied in petrolatum that was also used as a control test site. 72 hrs after patch testing, samples of the stratum corneum (SC), the uppermost epidermal layer, were collected from the test sites by using adhesive tapes. In the SC tapes, 15 cytokines of different signature (Th1, Th-2 and innate markers) were determined by a multiplex immunoassay. Next, filaggrin degradation products (NMF) as an indicator of skin barrier damage were measured. From 23 tested subjects, seven had negative reaction after patch testing to a single fragrance. Of 15 detected cytokines, seven cytokines showed significant difference between a hapten and petrolatum (CCL17, CXCL10, CCL22, CXCL8, CCL2 and IL-22). We compared the sub-group of subjects who did not have a positive reaction to a single fragrance with the

group who did show a positive reaction to both, mix and a single fragrance. A significant difference between these sub-groups was found for IL-16 and CXCL10. IL-16 has previously been shown as a biomarker to distinguish irritant from allergic reaction. In addition, a sub-group with negative patch test to a single fragrance showed decrease in NMF further supporting the hypothesis that the positive reaction to the mix was caused by an irritant reaction. This study confirms previous findings that IL-16 might be a suitable biomarker to distinguish irritant from allergic reaction. This might be of relevance in diagnostics of contact dermatitis and interpretation of allergen patch testing.

PS 1274 Assessment of Aflatoxin and Ochratoxin Exposure among Mothers and Children in Nepal

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Contamination of mycotoxins in human diets are unavoidable, especially in the developing world. Aflatoxin B_1 (AFB_1), the most potent mycotoxin, is classified by IARC as a Group 1 human carcinogen. Recent evidences have shown that exposure to AFB_1 links to adverse maternal and children's health effects in various low- and middle-income nations. Additionally, AFB_1 was found to be capable of crossing blood-placental barrier and can result in adverse developmental outcomes, including low-birth weight. Ochratoxin A (OTA) is a toxicologically significant mycotoxin and has been frequently found to contaminate many food items. In this study assessment of these two mycotoxins was conducted using biomarker approaches in serum samples collected from the AflaCohort Birth Cohort Study (2015-2019) in Banke, Nepal, where healthy pregnant women were enrolled, and the mother-child pairs were followed-up to examine the potential adverse effects of mycotoxins exposure to the mother and children. The results presented here consist of blinded analyses which utilizes the latest batch of serum samples collected from this study, which consisted of a total of 699 serum samples divided into two batches (n1=486, n2=213). In addition to measurement of serum AFB_1-lysine adduct to quantify AFB_1 exposure, the levels of serum OTA were analyzed to assess exposure to OTA. Overall, AFB_1-Lysine was detectable in 81.48% of samples from first batch and 93.43% of samples from second batch, whereas OTA was detectable in all serum samples in both batches. For the first batch, the mean, median, and geometric mean for serum AFB_1-lysine were 2.13 (SD: 7.20), 1.01 (q1: 0.58, q3: 1.81), and 1.15 (95% CI: 0.93, 1.38) pg/mg albumin, respectively, while those for serum OTA were 0.47 (SD: 2.15), 0.26 (q1: 0.17, q3: 0.38), and 0.27 (95% CI: 0.09, 0.45) ng/mL serum, respectively. For the second batch, the mean, median, and geometric mean for serum AFB_1-lysine were 2.98 (SD: 9.06), 1.30 (q1: 0.84, q3: 4.80), and 1.52 (95% CI: 1.19, 1.86) pg/mg albumin, respectively, while those for serum OTA were 0.51 (SD: 0.61), 0.38 (q1: 0.28, q3: 4.84), and 0.41 (95% CI: 0.17, 0.64), respectively. The samples from the second batch showed higher overall exposure levels for both AFB_1 and OTA compared to those from the first batch, which may reflect seasonal variations of dietary exposure. Further analyses will be conducted to evaluate the association between levels of mycotoxin exposure and children's health outcomes.

PS 1275 Identification of Predictive Biomarkers Associated with Antibiotic-Associated Nephrotoxicity in Cystic Fibrosis Patients Using a Kidney Microphysiological Device

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The longevity of individuals living with cystic fibrosis (CF) through successful treatments and lung transplantation has brought with it unforeseen problems and complications: one being the emergence of acute kidney injury from antibiotic treatment stemming from colonized pulmonary microbes in these patients. We have employed a kidney microphysiological device system (MPS)with cultured human proximal tubule epithelial cells (PTECs) to identify potential sensitive renal biomarkers arising from exposure to aminoglycosides and polymyxins commonly used to treat CF patients. Specifically, we exposed PTECs to various concentrations of Polymyxin B (PMB), Polymyxin E (colistin) and tobramycin for 72 hours and collected daily effluents as a source for nephrotoxicity biomarker discovery. Biomarkers associated with epithelial cell injury KIM-1 (Kidney Injury Molecule 1) and NGAL (Neutrophil Gelatinase-Associated Lipocalin), inflammation (soluble Fas Receptor, Fas Ligand and TNF RI) and apoptosis (caspase-cleaved cytokeratin 18 and intact cytokeratin 18) were chosen to evaluate kidney chip effluents by ELISA. We observed an antibiotic-specific biomarker response with elevations of KIM-1 in the effluents at 24 hours with PMB, increased NGAL and FAS concentrations at 24 hours with colistin exposure and increased NGAL concentrations with tobramycin. These data suggest that the mechanisms of PTEC nephrotoxicity associated with antibiotic exposure can produce antibiotic-dependent biomarker response patterns that reflect underlying cellular injury. Follow up analyses with PTEC RNA transcript (RNAseq) evaluations from these studies along with microRNA analyses of the effluents will provide additional information for the selection of biomarker candidates.

PS 1276 Next-Generation Sequencing Pipeline for Genome-Scale Analysis of Circulating microRNAs as Discovery Approach for Safety Biomarkers

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MicroRNAs are small non-coding RNAs that regulate gene expression in virtually all humans cells. Changes in tissue-specific microRNA expression patterns have been associated with a variety of human diseases. These changes reflect re-modelling of the biological pathways during disease onset and progression that make microRNAs attractive potential drug targets as well as sensitive and specific biomarkers. Tissue-specific microRNAs have been identified in various cell-free biofluids including plasma, serum, CSF and urine. The levels of tissue-specific microRNAs in liquid biopsies can be used for early detection of drug-induced organ injuries, such as miR-122 for liver[1], and offer a novel class of safety and toxicity biomarkers[2]. The Translational Safety Biomarker Pipeline (TransBioLine) IMI2 consortium aims to discover and qualify novel microRNA safety biomarkers for five organ systems (kidney, liver, pancreas, vascular and central nervous systems) by 2024. Furthermore, TransBioLine aims to characterize inter- and intra-individual variability of circulating microRNAs through investigation of several healthy volunteer populations. Next-Generation Sequencing (NGS) technology enables to analyze circulating microRNAs expression patterns in the biofluids and/or tissues of diseased and healthy individuals on a genome-scale level. However, small RNA sequencing suffers from potential biases (e.g. adapter ligation bias) and often lacks absolute transcript quantification. In order to establish a reliable biomarker discovery and quantification pipeline for circulating microRNAs, a systematic comparison of four commercially available small RNA library preparation protocols for NGS has been completed: QIAseq (Qiagen), CleanTag (TriLink Biotechnologies), RealSeq-Biofluid (Somagenics) and NEXTFLEX v3 (BIOO Scientific). The performance of the selected kits in terms of sensitivity, repeatability, ligation bias, usability and cost has been evaluated using RNA isolated from human plasma and liver tissue as well as miRXplore Universal Reference (Miltenyi Biotec). The total number of unique microRNAs identified in human plasma samples varied between the selected NGS protocols: 357 for QIAseq, 360 for CleanTag, 393 for RealSeq-Biofluid and 359 for NEXTFLEX v3. A proprietary panel of exogenous small RNA spike-in controls[3] has been developed for monitoring ligation bias and to enable absolute quantification of microRNAs levels and hence comparability of data across independent datasets. This work will allow establishment of a best-in-class NGS pipeline for microRNA biomarker discovery and quantification in liquid biopsies. *References 1. Howell, L. S., Ireland, L., Park, B. K. & Goldring, C. E. MiR-122 and other microRNAs as potential circulating biomarkers of drug-induced liver injury. Expert Rev. Mol. Diagn. 18, 47-54 (2018). 2. Schraml, E., Hackl, M. & Grillari, J. MicroRNAs and toxicology: A love marriage. Toxicol. Rep. 4, 634-636 (2017). 3. Lutzmayer, S., Enugutti, B. & Nodine, M. D. Novel small RNA spike-in oligonucleotides enable absolute normalization of small RNA-Seq data. Sci. Rep. 7, 5913 (2017).*

PS 1277 Identification of Biomarker Genes in LUHMES Neurons to Screen for Neurodegenerative Toxicants

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Neurodegenerative diseases result from environmental and genetic factors, yet the environmental causes for Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis, *etc*. have not been identified. In order to screen chemical libraries for candidate neurodegenerative toxicants we identified and validated biomarker genes that respond to a variety of toxicants in LUHMES neurons. Initially, LUHMES conditionally immortalized human dopaminergic neurons were treated with seven neurotoxicants and subjected to RNA sequencing. Twenty-four genes responded to multiple toxicants, including three metallothionein genes that were dynamically increased by all seven toxicants. Testing of additional toxicants in additional cell types established that *MT1-G* responded to diverse toxicants in LUHMES cells, in SH-SY5Y cells, and in non-neural cell lines such as HepG2 and HUVEC. Finally, we investigated the mechanisms of regulation that have been described for metallo-

thionein using LUHMES neurons. Inhibitor drugs for the Metal-Responsive Transcription Factor 1 (MTF1, inhibited by APTO253) and the oxidative stress responsive/NRF2 pathway (NRF2, inhibited by ML385) decreased transcriptional induction of *MT1-G* in LUHMES neurons in response to some toxicants such as $CuCl_2$. Other toxicants such as Ziram induced *MT1-G* in LUHMES neurons but were unaffected by these two inhibitors, suggesting a third pathway can regulate this gene. These studies indicate that the *MT1-G* gene is a sensitive detector of diverse cell stresses in the LUHMES neuron model and may be useful for primary screens of environmental toxicant libraries to identify the neurodegenerative toxicants.

PS 1278 Characterization of an Aryl Hydrocarbon Receptor Response in Whole Blood at the Single-Cell Level

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor that binds many different chemicals, including toxic compounds such as polycyclic aromatic hydrocarbons and halogenated dibenzo-p-dioxins. Activation of the AhR leads to upregulation of genes driven by specific sequences in their promoters called aryl hydrocarbon response elements (AHRE). Here we examine upregulation of classic AhR responsive genes in the whole blood of the laboratory mouse for their potential as biomarkers of exposure to AhR ligands. Using the prototype AhR ligand beta-napthoflavone (BNF), a non-toxic AhR agonist, and the C57BL/6J mouse, we observed the marked upregulation of AhR responsive genes aryl hydrocarbon receptor repressor (Ahrr) and Cytochrome P450 1a1 (Cyp1a1) over time, with maximal expression between 4 and 5 hours and almost complete reduction by 10 hours. We determined that the expression of the Ahrr was the more sensitive upregulated gene when using Next Generation RNA sequencing protocols. To improve the sensitivity of the approach, we hypothesized that certain cell blood types were more sensitive than others and tested that idea by multiple methods. Flow cytometry, magnetic bead separations, and single cell sequencing suggests that AhR target genes were differentially inducible in different blood cell types. We observed Ahrr being the most highly induced in CD4+ cells while Cyp1a1 was most inducible in Cd11+ cells. We also examined the pharmacokinetics via mass spec of the BNF disposition in the blood of the mice and demonstrate that levels of this ligands precede the induced mRNA expression and are reduced in keeping with the RNA expression data. In summary, these data support the use of RNA targets of the AhR as proxies of exposure to AhR ligands, but also demonstrates considerable caution should be employed as the time course is more adept at identifying short-term (recent) exposures.

PS 1278a The Function of lncRNA RPL34-AS1 in Esophageal Cancer Cells

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Studies have shown that the abnormal expression of lncRNAs contributes to specific changes in tumor, and lncRNAs have been known as key biomarkers for cancer diagnosis, cure and prognosis. This study is to explore the function and potential mechanism of lncRNA RPL34-AS1 in esophageal cancer cells. Based on screening esophageal cancer cohorts from TCGA database, we identified RPL34-AS1 as a functional lncRNA in esophageal cancer. We evaluated the expression of RPL34-AS1 by qRT-PCR in EC109 and EC9706 human esophageal cancer cell lines, and Het-1A human esophageal immortalized epithelial cell line, respectively. Gain- and loss-of-functional of RPL34-AS1in EC109 cells were achieved by knock-in (plasmid transfection) and knock-down (RNAi) techniques. The transfection efficiency of RPL34-AS1 was analyzed by qRT-PCR. To evaluate the effects of RPL34-AS1 on the biology behaviors of esophageal cancer cells, cell proliferation, apoptosis, cycle, migration and invasion were examined by CCK-8, Edu staining, flow cytometry and Transwell assay. The results showed that RPL34-AS1 was down-regulated in esophageal cancer cells. Compared with Het-1A, the expression level of RPL34-AS1 in EC109 cells was decreased significantly($P<0.05$). After transfection by RNAi and plasmid techniques, the relative expression level of RPL34-AS1 decreased to 0.21 and increased by 7855 folds ($P<0.05$), respectively. Gain- and loss-of-functional assays revealed that RPL34-AS1 inhibited EC109 cells proliferation, cycle progression, invasion, and migration and promoted apoptosis. In conclusion, our finding suggests that lncRNA RPL34-AS1 is a tumor suppressor and a potential therapeutic choice for esophageal cancer. *(This study is supported by National Nature Science Foudation of China 81573191, 81872588).*

PS 1279 Examining the Transgenerational Effects of Environmental Cues in *C. elegans*

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In sexually reproducing organisms, germ cell development is vital for the faithful transmission of the genome and epigenome across generations. Recent studies have shown that germ cell development is affected by different environmental toxicants, resulting in a decrease in germ cell health and number. Here, we examine and compare the transgenerational impact and mechanisms of two prevalent toxicants, the plastic chemical Bisphenol A and ethanol. Both have well-described impacts on the developing fetus; however, their effects on developing germ cells and subsequent generations are less explored. We analyze the transgenerational effects of both compounds in *Caenorhabditis elegans.* We hypothesize that exposure disrupts the epigenetic machinery in germ cells, causing changes in histone modifications, fertility defects, and germline dysfunction in a transgenerational manner. First, we show that BPA exposure causes a transgenerational two-fold increase in germline chromatin desilencing with $p\leq0.01$ coupled with a reduction and redistribution of histone H3K9me3 and H3K27me3. We show that the alteration of repressive histone levels is required for the observed transgenerational 43% increase in germline apoptosis with $p\leq0.01$ and 85% increase in embryonic lethality with $p\leq0.001$. An increase in apoptosis suggest possible perturbations in the germline checkpoint machinery. We show that BPA exposure transgenerationally induces untimely single-stranded DNA invasion with $p\leq0.001$, 2.5-fold increase in incorrect crossover formation with $p\leq0.001$, and 67% increase in missgregation of the X chromosome. To understand which checkpoint BPA perturbs to cause the increase in apoptosis, we used mutants of each checkpoint to rescue the effect. This revealed that BPA perturbs the synapsis checkpoint because a *pch-2* mutant decreased BPA induced apoptosis by two-fold with $p\leq0.0001$ but not the DNA damage checkpoint. Similar to that of BPA, ethanol exposure at human-relevant doses also causes transgenerational chromatin desilencing and germline dysfunction, although to a lesser extent than BPA's. This project identified BPA's and ethanol's transgenerational effect on the germline machinery and reproductive health. We hope to further understand how it induces germline dysfunction, carrying important implications for human reproductive health in the context of environmental exposures.

PS 1280 Synthetic Progestins and Progesterone Induce Similar Proliferative Transcriptomic Profiles in the T47D Breast Cancer Cell Line

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The use of the synthetic progestin, medroxyprogesterone acetate (MPA), during hormone replacement therapy (HRT) has been linked to increased risk of invasive breast cancer. Currently, the biological mechanism underlying this relationship is unknown. Synthetic progestins are a class of pharmaceutical compounds that bind the progesterone receptor (PR) and are predominantly used as birth control and during HRT. It is unclear whether the use of synthetic progestins other than MPA is associated with invasive breast cancer. The goal of this study is to understand whether breast epithelial cells have the same physiological and transcriptional response to endogenous progesterone and synthetic progestins. T47D cells, a human breast epithelial cell line, were grown in culture and exposed to endogenous progesterone (P4) and four common progestins: (1) MPA, (2) etonogestrel, (3) norethindrone, and (4) levonorgestrel. First, a dose response experiment was conducted to determine the effects on cell proliferation, as growth arrest is a hallmark of progesterone exposure. Next, gene expression changes were assayed at physiologically relevant doses for both P4 (200nM) and the progestins (2nM). To determine transcriptional response, Progesterone Receptor (PGR), as well as the progesterone response sentinel gene, Zinc Finger And BTB Domain Containing 16 (ZBTB16) were assayed across the time points 2, 4, 6, 8, 10,12 and 24 hours. Based on these results transcriptomic profiles were assayed at 10 hours. Transcriptomic data show that P4 and synthetic progestins elicit the same response, with 94% of the response being conserved. Additionally, the magnitude and directionality of these changes are also conserved. Enrichment analyses demonstrate that there is an increase in cancer signaling, as well as pathways important in breast cancer development including AMPK and ERK/MAPK signaling pathways. Furthermore, enrichment of transcriptional regulators revealed that beyond hormonal regulators such as Estrogen Receptor 1(ESR1) and PR, cancer specific transcription factor, such as TP53 were enriched, as was E2F4, which has been linked to P4 associated growth. Taken together, these data suggest that activation of PR is linked to cancer related transcriptomic profiles. These findings suggest a molecular link exists between the epidemiological observations associating hormonal birth control use and breast cancer.

1281 Comparison of Toxicogenomic, Epigenomic, and Apical Points of Departure for Ketoconazole in a Developmental Toxicity Study

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Toxicity testing paradigms for novel molecules take several years of investment and require extensive animal usage. There is promise that leveraging alternative techniques, such as toxicogenomics, may be able to replace traditional testing strategies for risk characterization while reducing animal use and maintaining protection for human health. The objective of this study was to generate data to support the use of a transcriptome-based point of departure (POD) for developmental and reproductive toxicity (DART) by comparing transcriptome (mRNA and miRNA) to traditional PODs. Ketoconazole, a fungicide with established embryotoxic and fetotoxic properties, was used for proof of concept in a rat developmental toxicity study. Ten rats/dose group were exposed daily to vehicle control or ketoconazole with seven dose levels ranging from 0.63 to 40 mg/kg/day from gestation day 6-21. On GD 21, embryotoxicity and fetotoxicity endpoints were evaluated, and whole-genome RNA sequencing was performed on liver and placenta samples. Benchmark dose lower confidence limit (BMDLs) were generated on apical endpoints and normalized expression data. NOELs were determined for epigenetic (miRNA) endpoints and apical endpoints that could not be determined by BMD. Dam apical BMDLs ranged from 0.4 to 23.7 mg/kg/day, with placenta histopathology as the most sensitive endpoint. Fetal apical BMDLs NOELs ranged from 6.3 to 20.4 mg/kg/day, with fetal body weight as the most sensitive effect. Dam liver and placenta transcriptome BMDLs were 0.7 and 1.8 mg/kg/day, respectively, which were similar to the most sensitive apical endpoint evaluated in the study. Liver and placenta epigenomic NOELs (0.63 and 2 mg/kg/day), were similar to transcriptomic and apical PODs. These data provide support that labor- and animal-intensive DART studies could potentially be replaced with streamlined studies that employ transcriptomic data, potentially by utilizing the liver as a sentinel organ, which would not only still be protective of human health but also reduce animal use.

1282 Gene-Specific Promoter Methylation of Lead Exposure Biomarker Genes in Environmental Lead-Exposed Children from Kabwe, Zambia

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Environmental exposure can alter epigenetic regulatory features, such as DNA methylation, and it is linked to impacts on human health. Among environmental contaminants, lead (Pb), a probable human carcinogen, is a prevalent toxic metal that can alter altered global and gene-specific DNA methylation and associated with various human diseases. One mechanism of Pb toxicity is its inhibition of δ-aminolevulinic acid dehydratase (ALAD) activity in heme synthesis. Another gene that is affected by Pb is the tumor suppressor gene p16, a key factor involved in the early stages of carcinogenesis. Kabwe town of Zambia is ranked among "The 10 World's most polluted places" because of the Pb-Zn mine in the town operated for almost a century (1902–1994). However, to date, no data exist regarding the influence of environmental lead exposure on DNA methylation level in children from Kabwe. Thus, we examined the frequency of hypermethylation in promoter regions of ALAD and p16 genes in the Pb exposed children using methylation-specific PCR. After obtaining permission from Ministry of Health, Zambia, blood samples (n =150) were collected at 5 clinics (3 near the dumpsite and 2 far from the dumpsite) from children aged 2 to 10 years old. Blood Pb levels (BLLs) were measured by Lead Care II analyzer. Genomic DNA was extracted using NucleoSpin Blood Kit and methylation conversion of genomic DNA was done using EpiTect Bisulfite Kit. The average BLLs were 24.0 ± 9.2 µg/dL for exposed area and 7.9 ± 4.5 µg/dL for unexposed area. Analysis of the profile of methylation in exposed area showed a significant methylation level for both ALAD and p16 genes. The methylation frequencies of ALAD CpG were 84.3% and 42.1% in exposed and unexposed areas, respectively and their associations with the risk of Pb poisoning was significant (adjusted OR = 7.84, 95% CI: 3.22-19.07; p<0.001). The incidence of p16 promoter hypermethylation for same area categories was 61.7% and 42.1%, respectively. In general, aberrant methylation of ALAD and p16 genes promoter region in the Pb exposed children might offer an effective means for earlier diagnosis of Pb poisoning.

1283 A Single Nuclei Analysis of Changes in Epigenetic Sensitivity to Chemical Exposure with Age

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It is a well-known that the epigenome in early development is particularly vulnerable to chemical exposure. There is relatively little known, however, about periods of sensitivity during the aging process. Understanding how sensitivity to chemical exposure changes with age is crucial for designing thorough risk assessments. To map epigenetic sensitivity with age we are performing single nuclei RNA sequencing in adult *C. elegans*. *C. elegans*' short lifespan facilitates aging experiments and many fundamental age-related pathways are conserved between *C. elegans* and humans. Previously, we used immunofluorescence to show that regulation of certain histone marks becomes more variable with age, suggesting that older organisms may be more vulnerable to epigenetic perturbations. Single nuclei sequencing will help us further understand gene expression changes with age and chemical exposure. To assess the quality of our nuclei dissociations we used fluorescent activated cell sorting on nuclei dissociated from transgenic *C. elegans* containing GFP tagged germline nuclei. We obtain about 30% germline nuclei, suggesting we will be able to detect changes in the germline transcriptome with age and chemical exposure. After performing sequencing on day two adult *C. elegans*, we obtained 32,099 reads per cell, 1,515 genes per cell, and 10,810 nuclei sequenced, indicating that we had the sequencing depth to gain a comprehensive view of adult *C. elegans* transcriptomic changes and communication between tissues. The breadth of our sequencing enabled us to obtain rare cell types, for example roughly 5% of our nuclei were neurons. However, we had a low sequencing saturation of 31%, suggesting our samples were overly concentrated. Next, we will expose *C. elegans* to two chemicals pervasive in the environment, bisphenol A and perfluorooctanesulfonic acid, at different ages and then perform single nuclei sequencing. Subsequent chromatin immunoprecipitation sequencing will identify the upstream epigenetic changes associated with the observed gene expression changes. We will compare perturbations to the transcriptome and epitranscriptome at different ages to identify changes in chemical sensitivity with age. One benefit of our approach is that we can evaluate transcriptomic changes in specialized cell types, such as neurons, whose expression patterns are usually masked in bulk RNA sequencing. Furthermore, our research will help fuel dialogue on how to account for age when designing chemical safety assessments.

1284 Somatic Expression of piRNA in the Mouse Identifies Short, Tissue-Specific piRNA: Potential Implications for Toxicoepigenetics

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Piwi-interacting RNAs (piRNAs) are a class of small non-coding RNA that associate with PIWI proteins for DNA methylation-induced retrotransposon suppression and are highly expressed from the germline. Mature germline piRNAs typically contains 24-32 nucleotides, 5′ uridine signatures, adenosine signatures at position 10, and 2′-O-methylation signatures at 3′ ends. Non-coding RNA expression is sensitive to environmental exposures, though the extent to which piRNA expression is modified by environmental toxicants is poorly understood. Traditionally, piRNAs were considered to be associated with sequence-specific *de novo* DNA methylation primarily of transposons and genic regions in germ cells. The current study overturns this accepted knowledge by revealing widespread PIWIL and piRNA expression from the mouse soma by incorporating qRT-PCR methods and next-generation sequencing of small RNAs enriched for piRNAs following sodium periodate treatment, respectively. Although *Piwil1*, *Piwil2*, and *Piwil4* were highly expressed in the germline, their somatic expression was also observed in the brain, liver, kidney, and heart. Sequencing results indicated short, tissue-specific somatic piRNA expression, with the hippocampus containing 5,494 piRNA-like peaks, followed by cortex (1,963), kidney (580), and liver (406). The study identified 26 piRNA sequence species and 40 piRNA locations exclusive to examined somatic tissues, compared to germline piRNA. Despite the lack of exposure-specific piRNA biomarker studies in the field of toxicology, cancer research has identified disease-associated piRNA biomarkers. As such, our ongoing research profiles the longitudinal tissue-specific effects of perinatal exposures on the piRNA transcriptome in mice, to reveal exposure-associated biomarkers in advancing disease interventions and therapeutics including ncRNA-based epigenome editing.

1285 Tissue- and Sex-Specific DNA Methylation Changes in Mice Perinatally Exposed to Lead

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Lead (Pb) is a well-known toxicant that can interfere with the development of a child's nervous and metabolic systems and increase the risk of developing diseases later in life. Although studies have investigated epigenetic effects associated with Pb exposure, knowledge of genome-wide changes with *in vivo* low dose perinatal Pb exposure in multiple tissues is limited. Within The Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET II) consortium, we utilized a mouse model to investigate tissue- and sex-specific DNA methylation. Experiments were performed using genetically invariant mice 93% identical to C57BL/6J. Dams were assigned to control (0 ppm) or Pb-acetate (32 ppm) water, resulting in average maternal blood lead levels below the limit of detection and 32 µg/dL, respectively. Exposures started two weeks prior to mating and continued until weaning at post-natal day 21 (PND21). One target tissue (adversely affected by exposure, i.e. liver) and one surrogate tissue (easily accessible, i.e. blood) were collected from PND21 mice (N=6 male and N=6 female per group), and the DNA methylome was assessed using enhanced reduced representation bisulfite sequencing (ERRBS). Differentially methylated cytosines (DMCs) and differentially methylated regions (DMRs) were identified using methylSig with a FDR<0.05 and a methylation difference >10%. We identified tissue-specific DMCs from male (n=426 and n=690) and female (n=514 and n=602) mice in liver and blood, respectively. Among DMC-associated genes, 19 and 12 of them overlapped between sex in blood and liver, respectively. In addition, we found 15 DMC-associated genes (e.g. *CACNA1I*, *PHACTR1*, *AIFM3*) that overlapped between blood and liver in males and a distinct set of 15 DMC-associated genes that overlapped in females. In parallel, we used 50 base-pair regions to identify tissue- and sex-specific DMRs. Tissue-specific DMRs in males (n=509 and n=326) and females (n=399 and n=369) were found in blood and liver, respectively. Overall, our findings demonstrate that low dose perinatal Pb exposure can induce tissue- and sex-specific DNA methylation changes and provide information for future Pb studies in humans.

1286 Preferential Tissue Hypomethylation of the Mouse Epigenome by Low-Dose Decitabine Exposure

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Decitabine (5-aza-2'deoxycytidine; DAC) is a small molecule drug commonly used to demethylate the epigenome in cell and whole animal models. However, current literature fails to identify the optimal dose of DAC to demethylate the epigenome. Additionally, many reports use imprecise measures of DNA methylation. Our preliminary data used reported doses in whole mouse models inducing high cytotoxicity with minimal to no changes in blood or somatic tissue DNA methylation. The current study aims to find the threshold of DAC to generate hypomethylation of the epigenome without causing cytotoxic effects. Mice were exposed to chronic low doses of decitabine ranging from 0 to 0.35 mg/kg over a period of 7 weeks. Testes function by weight and sperm concentration had dose response correlations along with gene expression within testes and liver, confirmed by qPCR and RNA seq. We find hypomethylation induced by DAC is locus (mLINE, IAP, IGF2, TCF3) and tissue (cortex, hippocampus, liver, testes, blood) specific, and is not uniform as previously reported. Bone marrow pathology indicates doses exceeding 0.35mg/kg during a chronic exposure prompts cytotoxic effects. Further, higher doses of DAC tend to hypermethylate rather than hypomethylate the epigenome by our WGBS analysis. Our findings indicate DAC is tissue specific, and may not be an ideal negative control for hypomethylation.

1287 Nonclinical Safety Assessment of Pharmaceutical Agents That Act Through Epigenetic Mechanisms of Action: Current Status and Industry Perspectives

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Pharmaceutic products designed to act as epigenetic modulators have been approved by regulatory authorities for treatment of advanced cancer. While the predominant effort in epigenetic drug development continues to be in oncology, nononcology indications are also garnering interest. A survey of pharmaceutical companies was conducted to assess the interest and concerns for developing small molecule direct epigenetic effectors (EEs) as medicines. Survey themes addressed the general levels of interest and activity with EEs as therapeutic agents, potential safety concerns, and possible future efforts to develop targeted strategies for nonclinical safety assessment of EEs. Thirteen companies contributed data to the survey. Overall, the survey data indicate the consensus opinion that existing ICH guidelines are effective and appropriate for nonclinical safety assessment activities with EEs. Special attention in the framework of toxicology study designs should be considered for delayed or latent toxicities, carcinogenicity, reproductive toxicity and the potential for transgenerational effects. While current guidelines have been appropriate for the nonclinical safety assessments of epigenetic targets, broader experience with a wide range of epigenetic targets will provide information to assess the potential need for new or revised risk assessment strategies for EE drugs.

1288 Epitranscriptomics of RNA Oxidation Reveals Mechanisms of Lung Toxicity and Disease

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Chemical modifications of proteins and nucleic acids play critical roles in the regulation of gene expression. New evidence suggests that environmental stresses may be involved in the misregulation of the functional activities of RNA modifications and pathways during lung distress and disease. Air pollution is the major source of environmental toxicity to the pulmonary cells. Components of air pollution can generate free radicals and perturb redox homeostasis in cells, leading to formation of the prominent RNA oxidative modification 8-oxo-7,8-dihydroguanine (8-oxoG). This mark is predominantly induced in respiratory cells, and it leads to genotoxicity as it preferentially mispairs with adenosine. Given the prevalence of RNA oxidation under environmental challenges, it is critical to identify novel molecular mechanisms involving RNA oxidation toxicity to cellular physiology and disease. To address this, we developed a high-throughput sequencing method to profile 8-oxoG in mRNAs of human bronchial epithelial BEAS-2B cells. Cells were exposed for 1.5 hours to realistic air pollution mixtures derived from the reaction of ozone (100 ppb) with acrolein (100 ppb), methacrolein (97ppb) and α-pinene (44ppb), common organic volatile compounds in the atmosphere. These compounds formed a multi-component gas-phase mixture and submicron secondary organic aerosols (PM 1.0) with concentrations ~ 40 µg/m^3. Using this approach, we found 42 transcripts that are consistently oxidized by air pollution. These transcripts belong to key oxidative stress, signaling, and metabolic pathways. Importantly, one of the mRNA transcripts that is more susceptible to oxidation is FDFT1, a protein that catalyzes a regulatory step in cholesterol synthesis and free-radical scavenging. Moreover, we confirmed a ~2-fold (p-value < 0.05) decrease in expression of FDFT1 protein, and a ~1.7-fold (p-value < 0.001) decrease in cellular cholesterol levels. To further validate the involvement of misregulation of cholesterol on cellular toxicity of air pollution, we knocked down FDFT1 using anti-sense siRNA in BEAS-2B cells. We found that FDFT1 knockdown promotes distinct morphological phenotypes typically observed in studies of lung stress and inflammation. Overall, our findings indicate that air pollution influences the formation of 8-oxoG marks in transcripts of epithelial lung cells leading to alterations on cellular function and cell morphology.

1289 Detrimental Effects of Flame Retardant PBB153 Exposure on Sperm and Future Generations

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In 1973, the Velsicol Chemical Company, which manufactured FireMaster, a brominated flame retardant, and NutriMaster, a nutritional supplement, mistakenly shipped hundreds of pounds of FireMaster to grain mills around Michigan where it was incorporated into animal feed and then into the food chain across the state. An estimated 6.5 million Michigan residents consumed polybrominated biphenyl (PBB)-laced animal products leading to one of the largest agricultural accidents in US history. To date, there have been no studies investigating the effects of PBB on epigenetic regulation in sperm, which could explain some of the endocrine-related health effects observed among children of PBB-exposed parents. Fusing epidemiological approaches with a novel *in vitro* model of human spermatogenesis, we demonstrate that exposure to PBB153, the primary component of FireMaster, alters the epigenome in human spermatogenic cells. Using our novel stem cell-based spermatogenesis model, we show that PBB153 exposure decreases DNA methylation at regulatory elements controlling imprinted genes. Furthermore, PBB153 affects DNA methylation by reducing *de novo* DNA methyltransferase activity at increasing PBB153 concentrations while reducing maintenance DNA methyltransferase activity at the lowest PBB153 concentration. Additionally, PBB153 exposure alters the expression of genes critical to proper human development. Taken together, these results suggest that PBB153 exposure alters the epigenome by disrupting methyltransferase activity leading to defects in imprint establishment causing altered gene expression, which could contribute to health concerns in the children of men exposed to PBB153. While this chemical is toxic to those directly exposed, the results from this study indicate that the epigenetic repercussions may be detrimental to future generations. Above all, this model may be expanded to model a multitude of environmental exposures to elucidate the effect of various chemicals on germline epigenetics and how paternal exposure may impact the health of future generations.

1290 Establishing Methods to Assess Epigenetic Signatures in Archived Study Tissues

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Revisiting archived studies using new methods on preserved samples will aid in a molecular understanding of adverse chemical effects, thereby reducing animal use, enhancing chemical hazard prioritization, and providing weight of evidence for regulatory decision making. Alterations in DNA methylation can occur with environmental chemical exposures, persist over time, and result in adverse health outcomes. In this pilot work, we tested the ability of Reduced Representation Bisulfite Sequencing (RRBS) to measure DNA methylation in formalin fixed paraffin embedded (FFPE) tissue samples relative to paired frozen controls. The 5 yr old liver tissue samples were obtained from male mice treated with 600 ppm phenobarbital (PB), a known mediator of DNA methylation changes and potential tumorigen, or vehicle control in drinking water for 28 d. Sequencing results indicate that FFPE samples contained significantly higher CpG sites relative to frozen samples (average 8.5 and 4.4 million reads, respectively with p=0.010; Kruskal-Wallis rank sum test); however, with higher read depth criteria (≥ 5X CpG site coverage), the remaining read counts for each sample type were statistically similar. In addition, read count variability in FFPE samples was significantly higher (p-value = 1.767e-11; Kruskal-Wallis rank sum test) than in frozen samples, overall indicating poorer quality sequencing data associated with FFPE. Only a few differentially methylated regions (DMRs) due to PB exposure were quantified in either sample type (4 and 1 DMRs, FDR p<0.05 for frozen and FFPE, respectively). When assessed with less stringency (uncorrected p<0.03 and methylation changes of >1%), 644 and 963 DMRs were identified for frozen and FFPE samples, respectively; however, only 30 DMRs were shared between sample types. In addition, genes linked to DMR regions overlapped less than 2% of previously published DMR genes due to short-term PB exposure in mouse liver, regardless of sample source. This indicates the study sample size (n=3 per condition) was of insufficient power to determine true DMRs. Follow-up studies in progress will address this issue. With sufficient power, we estimate that these FFPE-sourced data will accurately represent data from frozen/fresh tissue, thereby unlocking epigenetic-based data from archived tissue to refine mechanistic understanding of susceptibility to chemical-mediated toxicity. *This abstract does not necessarily reflect the policy of the US EPA.*

1291 Prenatal Lead (Pb) Exposure's Effect on DNA Methylation (5mC) and Hydroxymethylation (5hmC) in Adolescent Whole Blood

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Gestational lead (Pb) exposure can adversely impact offspring health through multiple mechanisms, one of which is alteration of the epigenome via DNA methylation, addition of a methyl group to the 5th carbon of cytosine, and hydroxymethylation, an intermediate in oxidative demethylation that is associated with increased gene expression. Most methods collectively measure 5-methylcytosine (5mC) and hydroxymethylcytosine (5hmC) without distinguishing between the two. The aim of this study was to identify the association of prenatal Pb exposures at each trimester (T1, T2, T3) with 5mC and 5hmC levels at multiple cytosine-phosphate-guanine (CpG) sites within candidate regions of genes previously associated with prenatal Pb exposure (*HCN2*, *NINJ2, RAB5A* and *TPPP)* in whole blood leukocytes of children ages 11-18 years. Participants from the Early Life Exposure in Mexico to Environmental Toxicants (ELEMENT) birth cohorts with archived whole blood were selected (n=144) for analysis by pyrosequencing following oxidative or standard sodium bisulfite treatment. Combined, this method directly quantifies total methylation (5mC+5hmC) and 5mC only; 5hmC is obtained by subtraction. Participants are 51% male with blood lead levels (BLL) at 3.29 ± 4.44 µg/dL during collection with historical T1 maternal BLL 6.43 ± 5.16 µg/dL, T2 BLL 5.66 ± 5.21 µg/dL, and T3 BLL 5.86 ± 4.34 µg/dL. 5mC levels were measured in *HCN2* (83.5 ± 8.87%), *NINJ2* (36.3 ± 23.0%), *RAB5A* (1.48 ± 1.30%), and *TPPP* (90.8 ± 13.2%). 5hmC levels were calculated in *HCN2* (2.05 ± 4.13%), *NINJ2* (2.32 ± 5.69%), *RAB5A* (0.67 ± 1.17%), and *TPPP* (1.11 ± 6.96%), providing evidence for 5hmC presence in whole blood. Controlling for sex, current BLL, and experimental plate, T1 maternal BLL was associated with 5mC in *NINJ2* (β=-3.85, p=0.00020) and *HCN2* (β=2.33, p=0.00054); T2 BLL was associated with 5mC in *HCN2* (β=2.27, p=0.025) and 5hmC in *NINJ2* (β=2.73, p=0.0073); and T3 BLL was associated with 5mC in *HCN2* (β=2.18, p=0.031) and 5hmC in *NINJ2* (β=3.52, p=0.00063). No statistical significance was identified in *TPPP* or *RAB5A.* Future studies need to quantify gene expression to determine if 5mC/5hmC changes are promoting transcriptomic alterations and investigate epigenetic patterns in other tissues.

1292 Prediction and Analysis of Hyper- and Hypomethylated Genes Associated with Breast Cancer

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Breast cancer is one of the major cancers affecting women around the world. Several reports showed that epigenetics plays a major role in cancers. Among the epigenetic modifications, DNA methylation (DNAm) is known to play a key role in breast cancer. In the present study, we aimed to analyze the epigenetic modifications in breast cancer among African Americans (AAs) and Caucasian Americans (CAs). To this end, we have downloaded the publicly available DNAm data for breast cancer among AAs and CAs from the TCGA database. Differential methylation analysis for the downloaded TCGA datasets was performed using *TCGAbiolinks*, an R/Bioconductor package for integrative analysis with GDC data. Results from 160 samples in AAs and 578 samples in CAs were available for breast cancer in TCGA database. Differential methylation analysis showed that there were statistically significantly differentially methylated positions (DMPs) between AAs and CAs. Among these DMPs, genes *MRPL28* (Mitochondrial Ribosomal Protein L28) and *PACS2* (Phosphofurin Acidic Cluster Sorting Protein 2) were identified as top five hypermethylated and the genes *RPH3AL* (Rabphilin 3A Like [Without C2 Domains]), *S100A14* (S100 Calcium Binding Protein A14) and *HOOK2* (Hook Microtubule Tethering Protein 2) were identified as top hypomethylated genes at a p-value <0.05. In conclusion, results from our study may provide further insights into the genes that influence the health outcomes in breast cancer among AA and CA populations.

1293 Morphine Sulfate Downregulates Mitotically Heritable Epigenetic Histone Modifications and Disrupts Pluripotency Phenotype in Human iPSCs

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Epigenetic (EG) manifestations and resulting genomic heterogeneity from opioid exposure is a novel and infrequent perspective to understand opioid induced toxicity. Aberrations in histone protein post-translational modifications (HP-PTMs) induce perturbations in chromatin integrity and subsequent differences in global gene expression patterns. In the current study, we hypothesized that morphine sulfate (MS) alters histone-3-protein (H3)-PTMs and pluripotency in human induced pluripotent stem cells (iPSCs). Observations of 21 global genomic H3-PTMs following exposure to 10 μM MS for 2 and 5 days suggest decreased levels of gene expression repressive H3-PTMs, H3K9me1 (2 days) and H3K27me3 (5 days). To confirm if these changes were reversible, cells were allowed to recover for 3 days in the absence of MS. Genomic levels of both H3K9me1 and H3K27me3 were not different from control, suggesting that MS induced EG effects are reversible. Furthermore, decrease in levels of H3K9me1 and H3K27me3 are concentration dependent and are not antagonized by pre-exposure of iPSCs to 10 μM naltrexone, suggesting an EG aberration in an opioid receptor independent pathway. Continuous MS exposure to 1 and 10 μM for longer durations rendered the levels of histones to initially level off, followed by another increase at 26 days, relative to control. Interestingly, shorter durations of MS exposure induced pluripotency as determined by stem cell specific nuclear transcription factor Oct-4 and cell surface marker TRA-1-60. Pluripotency stimulation was effectively inhibited when iPSCs were exposed to 10 μM naltrexone prior to MS exposure. However, except for a MS-induced decrease in TRA-1-60 following MS exposure for 29 days, pluripotency of iPSCs did not change. Together, the results indicate that MS alters pre-programmed H3-PTMs and stem-cell pluripotency according to opioid receptor independent/dependent mechanisms, respectively.

1294 Role of microRNA in Regulation of CD44 Involved in Development of Autoimmune Hepatitis

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Autoimmune hepatitis (AIH) is a rare severe liver disorder, which affects both children and adults worldwide. The incidence of AIH is increasing in the recent years. AIH may lead to develop cirrhosis and liver cancer. Studies have shown that AIH may result from the abnormal body's immune system, which attacks own liver, and causes inflammation and liver damage. However, the exact cause of AIH is unclear. Our study using concanavalin A (Con A)-induced AIH mouse model demonstrated that there was a significant increase in the plasma aspartate aminotransferase activity (AST) level, liver damage and liver-infiltrating mononuclear cells in CD44 KO mice when compared to C57BL/6 wild type mice. CD44 deficiency induced severe disease due to decreased apoptosis of liver-infiltrating cells and increased liver damage by such infiltrating cells. Our RNA sequencing data revealed that approximate 4,700 genes were up-regulated with a fold change >2.0, whereas 5,300 genes were down-regulated in liver tissues from Con A-induced AIH mice. Gene function analysis showed that the up-regulated genes such as BCL2L1, CASP3 and CASP8 in AIH mice might be responsible for hepatocyte apoptosis and liver injury, whereas the down-regulated genes such as IL-6 and SOCS1 in AIH might be associated with liver inflammation. Importantly, CD44 was up-regulated in AIH mice which could target BCL9 and β-catenin, suggesting that the expression and functions of CD44 may play a role in the regulation of Wnt/β-catenin signaling pathway, involved in autoimmune hepatitis pathogenesis. MicroRNA (miRNA) array analysis indicated that several miRNAs including miR-138-5p, miR-1911-3p, miR-1915-3p, miR-1249-5p, miR-1953 and miR-1356-3p, which could target CD44, were up-regulated in AIH mice; however, many CD44-targeting miRNAs such as miR-199a-3p, miR-328-3p, miR-471-3p, miR-483-3p, miR-708-5p and miR-1247-3p were down-regulated significantly in AIH mice. Interestingly, several up-regulated miRNAs such as miR-99a-3p, miR-503-3p, miR-6901-5p and miR-743a-5p could target β-catenin, suggesting that miRNAs may regulate Wnt/β-catenin signaling pathway and thus affect AIH pathogenesis. Moreover, the other up-regulated miRNAs such as miR-124-5p, miR-126a-5p, miR-137-3p and miR-140-5p could target CASP3, indicating that specific miRNAs may regulate cell apoptosis and thus affect autoimmune hepatitis pathogenesis. Further studies will be needed to elucidate the functions and underlying mechanisms of specific miRNAs in the regulation of CD44 involved in the development of autoimmune hepatitis. Our results suggest that CD44 may play an important role in the pathogenesis of autoimmune hepatitis. *Supported by NIH grants P01AT003961, P20GM103641, R01AT006888, R01ES030144, R01AI129788 and R01AI123947.*

1295 Epigenetic Regulation of Immune Response by Cannabidiol in Experimental Autoimmune Encephalomyelitis

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Several studies have suggested that ingredients in marijuana such as THC and CBD regulate immune response through epigenetic mechanisms. In this study we use microarray and next generation sequencing (NGS)-based approaches to determine how CBD alters histone/DNA methylation and expression of non-coding RNA (miRNA) and long non coding RNA (lncRNA) in CD4+ T lymphocytes in a mouse Experimental Autoimmune Encephalomyelitis (EAE) model. Treatment with CBD leads to attenuation in neuroinflammation and decrease in T cell proliferation in MOG induced EAE. We have found that the overall methylation levels of two histone marks, H3K4me3 which is associated with transcription activation and H3K27me3 which is associated transcription suppression, do not differ significantly in CD4+ T cells from naïve mice, EAE mice and CBD-treated EAE mice. However, histone methylation signal is enriched in the binding sites of certain transcription factors, such as ZNF143 and FoxA1, suggesting that these transcription factors may have important roles in CBD mediated immune modulation. In microarray study, we have found that the expression of protein coding transcripts as well as non-coding RNAs is altered in EAE mice compared to those in naïve mice. More importantly, the expression pattern of many transcripts is reversed by CBD treatment. A unique finding of this study is that the expression of many miRNAs and lncRNAs is dramatically affected by CBD. For example, increased expression of pro-inflammatory miR-155, -19, -210 and -223 in EAE is reversed by CBD treatment. Among those altered lncRNAs, most of them have not been validated experimentally and their functions are not known. We selected some validated lncRNAs and their expressions in CD4+ T cells from CBD and vehicle treated EAE mice are confirmed by real-time qPCR. For instance, lncRNA Neat1, which promotes cell proliferation and Mirt2, which inhibits the activation of NF-kB and MAPK pathways to limit the expression of proinflammatory cytokines are among the most significantly altered lncRNAs by CBD. In summary, this study shows that CBD regulates immune response through epigenetic mechanisms, particularly miRNA and lncRNA. *Supported by NIH grants P01AT003961, P20GM103641, R01AT006888, R01ES030144, R01AI129788 and R01AI123947.*

1296 PCB126-Induced Alterations in m6A RNA Modifications in Zebrafish Embryos

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Chemical modifications of proteins, DNA and RNA moieties play critical roles in regulating gene expression. Emerging evidence suggests these RNA modifications (epitranscriptomics) have substantive roles in basic biological processes. One of the most common modifications in mRNA and noncoding RNAs is N^6-methyladenosine (m6A). In a subset of mRNAs, m^6A sites are preferentially enriched near stop codons, in 3' UTRs, and within exons, suggesting an important role in the regulation of mRNA processing and function including alternative splicing and gene expression. Very little is known about the effect of environmental chemical exposure on m6A modifications. As many of the commonly occurring environmental contaminants alter gene expression profiles and have detrimental effects on physiological processes, it is important to understand the effects of exposure on this important layer of gene regulation. Hence, the objective of this study was to characterize the acute effects of developmental exposure to PCB126, an environmentally relevant dioxin-like PCB, on m6A methylation patterns. We exposed zebrafish embryos to PCB126 for 6 hours starting from 72 hours post-fertilization and profiled m6A RNA using methylated RNA immunoprecipitation followed by sequencing (MeRIP-seq), as well as assessing changes in mRNA splicing. We observed 6,983 and 11,315 m6A peaks in DMSO and PCB126 exposure groups (FDR 5%), respectively. The majority of the peaks are preferentially located around the 3'UTR and stop codons. Pathway analysis of m6A marked transcripts induced by PCB126 exposure revealed that these transcripts are associated with important developmental pathways (MAPK, Hedgehog, Notch and Wnt). These results suggest that PCB126 could affect developmental gene expression patterns by altering m6A levels. Gene expression analysis revealed upregulation of classical aryl hydrocarbon receptor (AHR) target genes such as cytochrome P450s in response to PCB126 exposure. Interestingly, none of the AHR target genes overlapped with the m6A altered transcripts, suggesting that xenobiotic metabolism may not be under m6A regulation. Further studies are necessary to understand the functional consequences of exposure-associated alterations in m6A levels. *This work is supported by NIEHS ES024915.*

1297 Single Cell Analyses Identify NK-Like T Cells in Peripheral Blood Affected by Tobacco Smoke Exposure

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Exposure from tobacco smoke has been found to impact immune response, leukocyte subtypes, DNA methylation, and expression from human whole blood. Analysis with single cell technologies will resolve smoking associated (sub)population compositions, gene expression differences, and identification of rare subtypes masked by bulk fraction data. Single cell RNA sequencing (scRNAseq) from primary peripheral blood mononuclear cells (PBMCs) identified shifts in subtype distributions within the CD4 T and CD8 T cell populations between smokers (n=4) and nonsmokers (n=4). In order to confirm and immunotype the PBMC populations, we used CyTOF with a 26-marker phenotypic panel to evaluate ~ one million viable cells from the same individuals profiled in scRNAseq. Major cell type population frequencies showed strong correlation between scRNAseq and CyTOF (Pearson r=0.99, p<0.0001). Using the VorteX Clustering Environment and the X-shift algorithm in combination with manual gating (Cytobank) for CyTOF analysis, we found no changes (Mann-Whitney, p=0.69; p=0.42) in the total number of CD8 T cells and Natural Killer T (NKT) cells, which was consistent with scRNAseq analysis. From the CD8 T cells, CyTOF found a significant increase (p=0.03) in the frequency of $CD16^+$ CD8 T cells in smokers. CD16 is commonly associated with nonclassical monocytes and Natural Killer (NK) cells. In NK cells, CD16 acts as a receptor that binds IgG antibodies to activate antibody-dependent cellular cytotoxicity (ADCC) processes. Using manual gating, the majority of this NK-like $CD16^+$ CD8 T cell subtype was CD45RA positive for both smokers and nonsmokers, indicating that this CD8 T cell subtype effector memory T cells re-expressing CD45RA (T_{EMRA}) cells. To demonstrate reproducibility and further phenotype smoking-altered T cell subpopulations, we designed a 36 antibody CyTOF panel containing markers to differentiate among naïve, central memory, effector memory and end-stage effector memory T subsets. We also quantified expression of cytolytic effector proteins (GZMB and PRF1) and exhaustion-associated proteins (PD-1, TIM-3, and CTLA-4).

1298 Epigenetic Signatures of the Past-Bisphenol A Exposure in Germ Line and Somatic Cells across Two Generations: A Transgenerational Study

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Exposure to environmental chemicals can have far reaching health effects especially when it occurs during the sensitive windows of embryonic development. Some chemical exposures have been found to be inherited by subsequent generations even after the exposure was ceased several generations ago. The effects that develop in absence of exposure but due to the past exposure of their ancestors is called transgenerational health effects. Transgenerational health effects in humans are not clearly understood; however, the studies from non-human studies suggest that chemicals can leave some exposure-specific epigenetic marks on germ stem cells that are transmitted to subsequent generations resulting in adverse health outcomes. The health effects developed at the multigenerational (in presence of direct contact with the chemical) seem to be different from transgenerational health effects. No epigenetic biomarkers have been identified that can reliably identify the past exposure and predict future health outcomes. The epigenetic marks are cell type and developmental stage specific. The transgenerational epigenetic marks that are resistant to epigenetic reprogramming are thought to be consistent across generations; however, very little is known about their specificity and role in development of predicted phenotypes. We are trying to identify epigenetic signatures of past bisphenol A exposure (10 ug/L) in medaka fish germline and somatic cells. We exposed medaka fish embryo from the day of fertilization to 12 days post fertilization and studied the dynamics of DNA methylation and gene expression across various stages of germ cells and across generations. Our results demonstrate that: a) the BPA-induced epigenetic marks escape epigenetic reprogramming (the process of erasure of parent-derived epigenetic marks and establishment of offspring cell/sex specific epigenetic marks) in germ stem cells (primordial germ cells, PGCs); b) some new epigenetic marks are established in germ cells during spermatogenesis in males; and c) majority of the BPA-specific epigenetic marks are *de novo* established past gametogenesis and are maintained by sperm. Approximately 30% reduction in fertility observed in males due to ancestral BPA exposure (past three generations). In this talk, we will present epigenetic marks of the past BPA exposure that are transmitted to offspring across two subsequent generations, and transcriptional pathways linked to these epigenetic alterations, which are potentially useful for prediction of adverse health outcomes. *Supported by fundings from National Institute of Environmental Health Sciences (NIEHS) R21ES027123, R21ES027123-02S1.*

1299 Arsenic-Induced Polyadenylation of Canonical Histone H3.1 mRNA Facilitates Cell Transformation through Inhibition of Variant Histone H3.3 Assembly

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Arsenic is the number one global carcinogenic contaminant, affecting more than 150 million people worldwide. Our previous study revealed that exposure to arsenic reduces cellular levels of the stem-loop binding protein (SLBP), which is critical for canonical histone pre-mRNA processing, resulting in the generation of polyadenylated H3.1 mRNA *in vitro*. This is intriguing because in general canonical histone mRNAs, unlike most other genes in multicellular organisms, do not possess a poly(A) tail. In the current study, we investigated the role of arsenic-induced H3.1 mRNA polyadenylation in carcinogenesis and the underlying mechanisms. The soft agar colony formation assay and xenograft assay demonstrate that ectopic expression of polyadenylated H3.1 mRNA promotes anchorage-independent cell growth and tumor formation in nude mice. Moreover, RNA-seq, flow cytometry cell cycle analysis, and chromosome spread assays display that polyadenylation of H3.1 mRNA causes transcriptional deregulation, G2/M cell cycle arrest, chromosome aneuploidy and aberrations. Notably, the ChIP-seq results reveal that induction of polyadenylated H3.1 mRNA results in displacement of the variant H3.3 from critical gene regulatory elements such as active promoters, insulators, and enhancers. H3.3 displacement appears to be critical for arsenic-induced carcinogenesis, since knockdown of H3.3 by siRNA induced cell transformation, whereas overexpression of H3.3 rescued cells from arsenic-induced transformation. Importantly, arsenic was capable of inducing high levels of H3.1 mRNA polyadenylation and marked loss of the SLBP in lung tissues of A/J mice exposed to arsenic (0, 100, and 200 μg/L) by inhalation for one week. This study uncovers polyadenylation of H3.1 mRNA and resulting displacement of H3.3 from regulatory elements as a potential mechanism for carcinogenesis. In addition, our findings add new insight not only into the oncogenic role for histone variant H3.3 but also into genomic instability induced by imbalance in histone stoichiometry. *This work was supported by National Institutes of Health grants R01ES026138, P30ES000260, R01ES029359, R01ES022935, and K22CA204439.*

1300 The Organophosphate Pesticide Methyl-Parathion Modulates the Expression of DNA Methylation-Demethylation Genes Through Oxidative Stress in Mice Testicular Cells

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DNA methylation-demethylation (DNA-M/D) is an epigenetic mechanism, which is associated with gene expression. DNA methylation involves the covalent transfer of methyl groups to cytosine from cytosine-guanine (CpG) sites, forming 5-methylcytosine (5-mC); this process is catalyzed by DNA-methyltransferases (DNMTs). Active DNA demethylation, catalyzed by ten-eleven translocation enzymes (TETs), consists in the sequential oxidation of 5-mC to finally be repaired to cytosine. The regulation of this process is not fully known; however, it has been proposed that reactive oxygen species (ROS; either endogenous or exogenous) can regulate DNA-M/D. The organophosphate pesticide methyl-parathion (Me-Pa), despite its highly toxicity, is employed in developing countries and it produces oxidative damage in macromolecules of sperm cells, as well as DNA alkylation. Recently, we reported that Me-Pa exposure generates promoter-specific hypermethylation in antioxidant response and DNA repair genes in sperm cells, but the mode of action is unknown. The objective of this study was to evaluate the *DNMTs* and *TETs* expression and methyl-purine DNA glycosylase (*MPG;* alkylation repair gene) expression in sperm cells of mice exposed to Me-Pa (6 mg/kg/day/5 days) and co-exposed with Me-Pa (same dose)-Vitamin E (50 mg/kg/day/5 days) to evaluate ROS participation. The exposed group to Me-Pa showed an increase in *DNMT3B* (*de novo* methylation), *DNMT1* (maintenance of methylation), *TET1*, and *TET2* expression and a decrease in *MPG* expression. On the other hand, the co-exposed group showed lower expression of *DNMTs* and *TETs* genes comparing with Me-Pa treated animals, suggesting that ROS may modulate the expression of DNA-M/D genes. Moreover, we observed an increased in *MPG* expression in the co-exposed group, suggesting that Me-Pa could be generating direct methylations in DNA and thereby activating the alkylation repair. In summary, these results suggest that the modulation of DNA-M/D genes expression, which was related to oxidative stress generated by Me-Pa exposure may partially explain the promoter-hypermethylation and low expression of antioxidant and DNA repair genes previously observed after Me-Pa exposure.

1301 Understanding Environmental Impact on Germ Cell Differentiation Trajectories Using scRNA-Seq

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While the intricate steps of germ cell development have long been hypothesized to provide unique windows of sensitivity to environmental insults, little is known of the effect of chemical exposure on the epigenome of germ cells and of the mechanisms of inheritance of these effects. This gap is particularly significant as embryonic germ cells undergo an extensive remodeling of their chromatin which includes genome-wide demethylation and the establishment of a complex pattern of histone modifications. The failure to properly regulate these histone marks leads to spurious repetitive element expression, germ cell death and infertility. Here, we use *in vitro* generated mouse and human germ cells termed Primordial Germ Cell-Like Cells (PGCLCs), to conclusively show that their epigenetic remodeling period constitutes a period of high sensitivity to environmental exposure. To this aim, we exposed their precursors, epiblast-like cells (EpiLCs) as well as PGCLCs for various lengths of time to (1) known chemical inhibitors of specific histone marks and (2) a model environmental chemical: BPA. We examined the impact of these exposures on survival/viability as well as the levels of repressive histone marks such as H3K9me3 and H3K27me3. By using targeted chemical inhibitors, we show that the tight regulation of both repressive histone marks H3K9me3 and H3K27me3 is essential for survival of differentiating PGCLCs. We also show that low BPA concentrations (1 and 10uM) disproportionately affect PGCLCs survival but not EpiLCs. These effects correlate with a marked decrease of both repressive marks H3K9me3 and H3K27me3. Finally, by using scRNA-seq, we show that exposure of human PGCLCs to low BPA levels doses alters the developmental trajectory of PGCLCs and affects precursors of PGCLCs disproportionately compared to PGCLCs themselves. We expect this research to provide a much-needed examination of the pathways implicated in the sensitivity of early germ cells to environmental insults and at the root of infertility.

1302 Genome-Wide DNA Methylation Changes in MutaMouse Lung and Liver Tissue following Subchronic Exposure to Benzo[*a*]pyrene

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Benzo[*a*]pyrene (B[a]P), a representative polycyclic aromatic hydrocarbon and Group 1 human genotoxic carcinogen, is found in coal tar, tobacco smoke, indoor and outdoor air, and broiled meats. Accumulated evidence indicates that, in addition to having exposure-induced genotoxic effects, genotoxic carcinogens such as B[a]P can induce epigenetic alterations associated with carcinogenesis. It was previously reported that subchronic MutaMouse exposure to B[a]P resulted in the greater level of DNA adducts in the lungs, a target organ for B[a]P carcinogenicity in mice, relative to livers, a non-target organ; however, the frequency of induced *lacZ* mutations was comparable in both organs. The goal of this study was to investigate the effect of B[a]P exposure on epigenetic alterations in MutaMouse lung and liver tissue following subchronic exposure. Adult male MutaMouse were treated with 75 mg B[a]P/kg body weight/day via oral gavage for 28 consecutive days, and the status of DNA methylation investigated by reduced representation bisulfite sequencing (RRBS). Exposure to B[a]P produced a comparable number of treatment-related differentially methylated regions (DMRs) in both organs. A combined analysis of gene-specific DNA methylation and differentially expressed genes (DEGs) showed that among 3390 DMRs and 368 DEGs in the lungs and 3007 DMRs and 315 DEGs in livers, only 32 and 20 genes in the lungs and livers, respectively, displayed an inverse correlation between DNA methylation and gene expression. Despite there being no differences in the number of treatment-related DMRs in the lungs and livers of B[a]P-exposed mice, there were marked differences in the pattern of organ-specific DNA methylation. Treatment with B[a]P resulted in a profound loss of genome-wide DNA methylation in the lungs, an epigenetic carcinogenesis-related event associated with genomic instability. This was evidenced by a 2-fold greater number of hypomethylated DMRs than hypermethylated DMRs. In contrast, no substantial difference between the number of hypomethylated and hypermethylated DMRs was found in the livers. The results indicate that, in addition to genotoxic alterations, the carcinogenic effect of B[a]P may be associated with both cancer-related genotoxic and epigenetic alterations in the target organ.

1303 Low-Level Sodium Arsenite Exposure Decreases Histone H3 Lys36 Trimethylation in Human Liver HepaRG Cells

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Inorganic arsenic exposure is a worldwide environmental health problem. The underlying mechanisms of arsenic toxicity and tumorigenicity are still not completely clarified; however, the involvement of epigenetic alterations has been suggested in the arsenic-induced tumorigenic process. Previously we showed that exposure of mature liver HepaRG cells to a low concentration of sodium arsenite for 14 days induced prominent gene expression and genome-wide DNA methylation alterations. Here, we investigated the effect of inorganic arsenic on histone modifications in differentiated HepaRG cells. Treatment of HepaRG cells with sodium arsenite for the 14 consecutive days resulted in a marked decrease of global histone H3 lysine 36 trimethylation (H3K36me3). Mechanistically, the loss of H3K36me3 in sodium arsenite-treated cells was accompanied by down-regulation of the histone methyltransferase *SETD2* gene, which is involved in histone H3K36 trimethylation. In addition to a global loss of histone H3K36me3, the sodium arsenite treatment caused an extensive reduction in gene-specific histone H3K36me3, as detected by ChIP-seq analysis. Interestingly, 69.5% of gene-specific histone H3K36me3 peaks were in the exon/intron regions, while only 1.4% was associated with gene promoters. Furthermore, by using combined gene expression and ChIP-seq analysis, we identified 203 genes, which expression positively correlated with histone H3K36me3 enrichment. The expression of most of these genes, 160 genes or 79%, was associated with the loss of histone H3K36me3. These genes belong to several pathways, crucial for cellular function and homeostasis, among which were: lipid homeostasis, xenobiotic, bile, carboxylic acids, steroid metabolism, and epithelial to mesenchymal transition. These findings emphasize the importance of epigenetic alterations as one of the key molecular events in the pathogenesis of arsenic-associated tumorigenesis.

1304 Scoping Review of Polycyclic Aromatic Hydrocarbons (PAH) Human and Experimental Animal Cancer Studies

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The carcinogenicity of some, but not all, PAH are known. To gauge feasibility of a potential systematic review of PAH cancer hazard, we conducted a systematic literature search of PAH human and animal cancer studies. We screened studies and categorized exposure and cancer exanimated. Study quality was not evaluated. Human cancer studies included studies with occupational exposure, environmental exposure, and biomonitoring. Occupational studies usually assessed PAH exposure via expert developed job exposure matrices or expert classified exposure, and some studies used semiquantitative exposure matrices created from measured workplace ambient air. Lung cancer was reported on most; bladder, breast, pancreatic, laryngeal, prostate, and childhood brain cancers were also reported. Environmental studies used PAH in ambient air, traffic pollution, and household dust for exposure and reported on childhood leukemia and breast cancers. Studies of PAH urinary biomarkers reported on lung and breast cancers, while many PAH DNA adduct studies, some including gene-environment interaction, reported on lung, breast, and liver cancers. Measurements of individual PAH can be used as surrogates of PAH mixture, the real life human exposure. Multiple exposure types with overlapping cancer endpoints reported will be a strength in future assessments. Animal cancer studies were searched for PAH in general and 35 specific PAH names, which did not include PAH listed in the current Report on Carcinogens. Animal carcinogenicity, co-carcinogen, or initiation/promotion studies, published in English, with some physiologically relevant exposures (oral, dermal, inhalation, intratracheal instillation, or i.p. injection) were included. Nearly all studies were published before the year 2000. Among 24 PAH categorized, chrysene, benzo[*e*]pyrene, cyclopenta[*cd*]pyrene, pyrene, and anthracene were the most studied. Most studies were in mice with dermal application, and many reported skin neoplasms. Lung and liver, but not skin, tumors were reported in i.p. studies. Other exposure routes or report on other tumor sites were rare. Some dermal and non-dermal exposure studies reported no increase in tumors, while anthracene, benzo[*ghi*]perylene, chrysene, cyclopenta[*cd*]pyrene, fluoranthene, and pyrene had increased tumors at two or more sites. While most studies being on skin cancer of dermally exposed mice is a limitation, the highly similar design can be a strength in comparing PAH.

1305 Carcinogenic Susceptibility Comparative Study on rasH2 Mice Produced by Two Breeding Facilities

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CByB6F1-Tg(HRAS)2Jic mice (rasH2 mouse) are produced at two breeding facilities, CLEA Japan, Inc. (Fuji, Shizuoka, Japan) and Taconic Bioscience (Germantown, NY, USA), and are supplied worldwide. The rasH2 mouse produced from cross-breeding by BALB/cByJJic female and C57BL/6JJic-Tg(HRAS)2Jic hemizygous male, in order to prevent genetic drift, both CLEA and Taconic colony has been renewed within 10 generations by rederivation from frozen embryos preserved in the Central Institute for Experimental Animals (CIEA, Kawasaki, Japan). After the breeding colonies renewed, we regularly perform a carcinogenic susceptibility study to confirm the homogeneity of rasH2 mice produced by the two facilities. In this study, we investigated the carcinogenic susceptibility of rasH2 mice derived from the breeding colonies replaced in 2016-2017. A total of 120 mice obtained from the two facilities (60 from each colony) were divided into vehicle (citrate buffer) and MNU (*N*-methyl-*N*-nitrosourea, 75 mg/kg) groups. Fifteen mice of each sex were assigned to each group, injected intraperitoneally with vehicle or MNU (week 0), and observed for 26 weeks. When mice were identified as moribund during the study period, they were sacrificed, and almost all organs were investigated histopathologically. The survival rates of the MNU and Vehicle group at week 26 were 0.0-6.7% and 86.6-93.3%, respectively. In MNU group, the incidence of forestomach papilloma/squamous cell carcinoma, typical MNU-induced tumors, was 100.0% in mice produced at two facilities. The incidence of malignant lymphoma of mice produced at CLEA and Taconic were 73.3-93.3% and 66.7-73.3%, respectively. Lung adenoma and skin papilloma/keratoacanthoma, major MNU-induced tumors in this strain, were observed in several mice in the MNU groups from both facilities. No significant differences were found in the tumor incidences between the two facilities. Consequently, Carcinogenic conformity of rasH2 mice derived from the replaced breeding colonies in two facilities was confirmed in the present study. Furthermore, as a result of comparison of tumor incidences in the similar study performed in past, we judged that the carcinogenic susceptibility of the rasH2 strain has been well maintained for more than two decades.

1306 Evaluation of Dietary E171, a Food Grade TiO_2, on the Rat Intestine

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E 171, a food grade titanium dioxide (TiO_2), is a common additive in foods. We evaluated its possible effects on the gastrointestinal tract administered as 0, 40, 400, or 5,000 ppm of the diet for 100 days following pretreatment with IP injection of dimethyl hydrazine (DMH) or with vehicle only. There was no effect on survival, body weight gain or food or water consumption. Histopathology of the small intestine, spleen, liver, lungs, and testes showed no differences between groups. Large intestinal tumors were diagnosed in a few rats pretreated with DMH (one rat each in DMH+control, DMH+low dose, and DMH+mid-dose groups). Otherwise there were no histologic abnormalities of the large intestines. Aberrant crypt foci (ACF) were not significantly increased in animals fed E171 either alone or after DMH administration. As expected, there was an increase in ACF in rats pretreated with DMH compared to those not pretreated. Furthermore, there was no increase in goblet cell numbers per gland, length of intestinal glands, or goblet cells per unit length of glands in the distal colon. Our results indicate no effect of food grade E171 on the rat intestine whether administered alone or after pretreatment with DMH. This supports the lack of carcinogenic activity reported in a NCI 2-year bioassay involving dietary administration of TiO_2 up to 50,000 ppm.

1307 Cancer Risk Estimates for Carcinogenic Nitrosamine Contamination in Drugs

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A number of nitrosamines are recognized as potent genotoxic carcinogens, and more than 30 nitrosamines are listed as carcinogens under California's Proposition 65. Nitrosamines are present in tobacco smoke, and may occur as environmental contaminants in water, food, and personal care products. Nitrosamine contamination of several commonly used drugs, such as histamine-2 blockers and angiotensin II receptor blockers, has prompted several recent US FDA recalls. Nitrosamines identified in these recalled drugs include NDMA (N-nitrosodimethylamine), NDEA (N-nitrosodiethylamine) and NMBA (N-nitroso-N-methylamino butyric acid). Both NDMA and NDEA are classified as probable human carcinogens by IARC. NDMA is one of 13 N-nitrosomethyl-N-alkylamines (NMAs) that are listed as carcinogens under Proposition 65. NMBA is a metabolite of N-nitrosomethyl-N-butylamine (NMA-C4), one of the 13 NMAs carcinogens. Carcinogenic evidence of the NMAs is summarized briefly, including animal bioassays, genotoxicity and structure activity comparisons. Positive findings from genotoxicity and DNA adduct studies indicate that NMAs are likely to operate through genotoxic mechanisms. Structure activity comparisons with carcinogenic N-nitrosodialkylamines reveal common genotoxic and tumorigenic activities, with shared target tumor sites amongst chemicals and test animal species. For the cancer risk estimation, we applied the maximum concentrations of contaminated NDMA (20.2 µg per tablet) and NDEA (1.3 µg per tablet) in these drugs to assess the chemical-specific cancer risk. Higher cancer risk is associated with patients taking the drugs with NDMA contamination than with NDEA contamination. For patients taking the drugs with maximum NDMA dose per tablet every day for four years, the cancer risk was estimated to be 29 cancer cases per 100,000 patients. Using the same exposure scenarios, the cancer risk associated with NDEA contamination is estimated to be four cancer cases per 100,000 patients.

1308 Urinary Bladder Carcinogenicity of Acetoaceto-*o*-Toluidide in F344 Rats

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Recent epidemiological studies have indicated that occupational exposure to the aromatic amine acetoaceto-*o*-toluidide (AAOT) was associated with a marked increase in urinary bladder cancers in Japan. However, little is known about the carcinogenicity of AAOT. To evaluate the urinary bladder carcinogenicity of AAOT, male and female F344 rats were treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) for 4 weeks followed by dietary administration of 0, 0.167, 0.5, or 1.5% AAOT for 31 weeks. The incidences and multiplicities of bladder tumors were significantly increased in the 0.5 and 1.5% groups of male and female rats in a dose-response manner. AAOT and 7 downstream metabolites were detected in the urine of the male and female rats administered AAOT with levels increasing in a dose-dependent manner. The most abundant urinary metabolite of AAOT was the human bladder carcinogen *o*-toluidine (OTD), which was at least one order of magnitude higher than AAOT and the other AAOT metabolites. In a second experiment, male F344 rats were administered 0, 0.167, or 1.5% AAOT for 4 weeks. Cell proliferative activity, and gamma-H2AX expression, which is a novel marker for the prediction of carcinogenicity, were significantly increased in a dose-dependent manner in the bladder urothelium of rats administered AAOT. Gene expression analyses revealed that expression of JUN and its downstream target genes were increased in the urothelium of male rats treated with 1.5% AAOT. These results demonstrate that AAOT promotes BBN-induced urinary bladder carcinogenesis in rats and suggest that overexpressed of JUN and its downstream target genes may be involved the bladder carcinogenicity of AAOT. In conclusion, AAOT, like other carcinogenic aromatic amines, is likely to be a carcinogen to the urinary bladder, and OTD metabolized from AAOT is the ultimate carcinogen.

1309 Glyphosate Induces Peripheral Blood Abnormalities and Plasma Cell Neoplasms Resembling Multiple Myeloma in Mice

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Glyphosate is the most commercially successful and the most widely used herbicide in the United States and worldwide. It represents about 50% of United States herbicide use at ~250 million pounds annually; the annual use of glyphosate worldwide is ~1.8 billion pounds. There has been considerable debate around the carcinogenicity of glyphosate. The International Agency for Research on Cancer labeled glyphosate as "probably carcinogenic to humans". However, other agencies like the Environmental Protection Agency (EPA) have maintained that glyphosate is unlikely to pose a carcinogenic risk. In epidemiological studies on the association of cancer and glyphosate, only multiple myeloma (MM) and non-Hodgkin lymphoma have a positive and statistically significant association with glyphosate. In the present study, we employed the Vk*MYC mouse model of MM to assess the impact of glyphosate exposure on MM pathogenesis. Glyphosate induced splenomegaly and multiple peripheral blood abnormalities, including anemia and high serum immunoglobulin G (IgG) levels. In addition, glyphosate triggered bone lytic lesions and kidney damage in Vk*MYC mice. Even in wild-type C57BL/6

mice, glyphosate increased serum IgG levels, induced anemia, and increased plasma cell presence in the spleen and bone marrow, hallmarks of benign monoclonal gammopathy. Lastly, glyphosate upregulated the expression of the activation-induced cytidine deaminase (AID) in the spleen and bone marrow of both C57BL/6 and Vk*MYC mice. AID is a B-cell genome mutator and a key pathogenic player in MM and non-Hodgkin lymphoma. These data indicate that glyphosate accelerates monoclonal gammopathy development and promotes progression to MM in genetically-predisposed mice. This work offers the first direct experimental evidence establishing glyphosate as an environmental risk factor for monoclonal gammopathy of undetermined significance and MM. It reveals the underlying mechanisms of glyphosate-mediated MM pathogenesis and potentially benefit over millions with MGUS worldwide. This study also directly contribute to the science needed to inform EPA and other agency regulations of current and emerging glyphosate-based herbicidal products.

PS 1310 Differential Gene Expression in Bladder Tumors from Arylamine-Exposed Workers

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Bladder cancer is the sixth most common type of cancer in the United States. Although smoking is the most common risk factor, occupational exposure to benzidine and β-naphthylamine have been shown to increase bladder cancer risk 40-100 fold. Benzidine and β-naphthylamine are aromatic amines that were used in the production of azo dyes predominantly for paper, textile and leather industries. To our knowledge, there have not been any toxicological studies in cancer epidemiology with a case-case study design, comparing the molecular signatures of tumors from people with exposure to chemical carcinogens to those from people without such exposure. Here we compare gene expression in 22 formalin-fixed paraffin-embedded (FFPE) bladder tumors from people with high-level occupational exposure to arylamines to that in 26 FFPE bladder tumors from people without such exposure. Our study subjects were from a cohort of workers employed at the DuPont Chambers Works (Deepwater, NJ), a major producer of β-naphthylamine and benzidine until the 1960s. Bladder tumors from unexposed subjects were from the Roswell Park Cancer Institute (Buffalo, NY). The tumor samples comprised of old, hard-to-extract fixed tissue ribbons from surgeries between 1950–1990. RNA was extracted from these ribbons and was analyzed for fragment sizes. All of the 48 samples contained RNA fragments with a minimum length of 300 nucleotides (nt), essential for reliable quality determination of FFPE-derived RNA. Samples were normalized to contain 200 ng of 300 nt or longer RNA fragments. Gene expression was analyzed on Nanostring nCounter system using two panels, PanCancer Progression panel and a custom-panel. The analysis comprised of 770 cancer progression-related genes and 69 custom-designed arylamine- and bladder cancer-related genes. Preliminary analysis showed a differential gene expression pattern in bladder tumors from arylamine-exposed versus unexposed subjects. Further investigation is underway to estimate arylamine-associated gene enrichment and pathway scoring. These findings would provide insights into the mechanisms underlying pathologies associated with arylamine exposure.

PS 1311 Evidence Relating to the Carcinogenicity of Acetaminophen in Human Epidemiological Studies and Long-Term Animal Bioassays

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Acetaminophen is one of the most widely used analgesic and antipyretic drugs and is available in many prescription and over-the-counter medications. Concerns over the carcinogenicity of acetaminophen arose because it is the major metabolite of phenacetin, a recognized carcinogen that causes cancer of the renal pelvis. Acetaminophen has been classified as 'not classifiable as to its carcinogenicity to humans' with inadequate epidemiologic evidence in both of the previous reviews by the International Agency for Research on Cancer (IARC) in 1990 and 1999. Numerous studies have been published since. OEHHA reviewed the complete peer-reviewed, published literature on the carcinogenicity of acetaminophen. For the human epidemiology studies, the strongest evidence was for kidney cancer (renal cell carcinoma and cancer of the renal pelvis); the majority of these epidemiologic studies reported risk estimates above one, although not all were statistically significant. Positive dose-response trends were also observed. Several studies controlled for important confounders and a few assessed exposure through databases of prospectively collected prescriptions, minimizing the possibility of information bias. For lymphohematopoietic cancers as a group, the majority of epidemiologic studies reported positive associations, some statistically significant, with acetaminophen use. The results were the most consistent for leukemia/lymphoma combined, myeloid leukemias (including AML), NHL, and multiple myeloma. For liver cancer, positive associations were reported in the two well-conducted large cohort studies, although one study could not control for alcohol and tobacco use. For cancers of the breast, ovary, uterine endometrium, and prostate, the association with acetaminophen use was either decreased or null. The data for a number of other cancer sites were either sparse (e.g., brain, respiratory tract, gastrointestinal tract, pancreas, cervix, and all cancers combined) or inconsistent (e.g., skin and colorectum). In long-term animal carcinogenicity studies, tumor findings were observed in three of 10 studies in mice and three of seven studies in rats. Liver tumors were observed in male and female mice and rats, pituitary gland tumors were observed in female mice, bladder tumors were observed in male and female rats, and mononuclear cell leukemia was observed in female rats.

PS 1312 Exploring the Potential Carcinogenic Activity of Per- and Polyfluorinated Alkyl Substances (PFASs) Using High-Throughput Toxicity Screening Data

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Per- and polyfluorinated alkyl substances (PFASs) are ubiquitous, persistent and toxic chemicals that pose public health concerns. More than 4,700 PFASs exist; however, current scientific understanding of the toxicity of these compounds is informed by studies of a select few, primarily perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Carcinogenicity concerns have arisen based on limited evidence from epidemiological studies for PFOA and animal tumor findings and mechanistic data for PFOA and PFOS. We explored the carcinogenicity potential of other PFASs using the computational, high throughput screening tool, the US EPA CompTox Chemical Dashboard's ToxCast (https://comptox.epa.gov/dashboard). ToxCast screening has produced toxicity data for thousands of chemicals, including a number of PFASs. A number of the ToxCast assays can be mapped to the IARC ten key characteristics of carcinogens (Smith et al., *EHP*, 124:713, 2016). Results for PFASs screened in the ToxCast assays and visual analytics software, Toxicological Prioritization index (ToxPi, http://toxpi.org/) were used to describe the carcinogenic potential of PFASs. A comprehensive literature search was also conducted to check the consistency of our analyses with other mechanistic data streams. Twenty-three PFASs that had sufficient ToxCast data and covered a range of structural subclasses were selected for analysis. PFASs were active in several of the carcinogenicity realms identified by IARC, the most significant being receptor-mediated effects, oxidative stress induction and alterations in cell proliferation. Patterns observed varied by length of fluorine-bonded chains and/or functional group within and between each key characteristic, suggesting structure-based variability in activity. A vast range of biological perturbations suggested that PFASs are potentially active in a number of mechanistic pathways associated with carcinogenic potential. In general, the key characteristics associated with the ToxCast findings are also supported by literature from other mechanistic data streams. Additionally, we explore how this approach might be integrated in a read-across context to infer carcinogenicity for a subset of the data-poor PFASs.

PS 1313 Cotinine, a Major Nicotine Metabolite, Induces Cell Proliferation in Urothelium *In Vitro* and *In Vivo*

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Tobacco smoking is a major risk factor for various human cancers including urinary bladder carcinoma. In a previous study, nicotine enhanced urinary bladder carcinogenesis using a rat two-stage model. In the present study, we examined the effects of cotinine, a major metabolite of nicotine, on urinary tract carcinogenesis. Cotinine induced cell proliferation in bladder cancer cell lines. By western blot analysis, cotinine induced phosphorylation of Stat3 and expression of cyclin D1 in UMUC3 cells. The cell proliferation induced by cotinine was blocked by inhibitors of both nicotinic receptors and Stat3. In an *in vivo* study, cotinine in drinking water also induced cell proliferation and simple hyperplasia in urinary bladder and renal pelvis urothelium of rats, but to a lesser degree compared to nicotine. Cytotoxicity detected by scanning electron microscopy and apoptosis evaluated by caspase immunohistochemistry in the bladder urothelium were induced by nicotine but not cotinine.

These data suggest that cotinine enhances urothelial cell proliferation both *in vitro* and *in vivo*, and cotinine is considered to be partly involved in urinary tract carcinogenesis by nicotine.

1314 Comparative Toxicity and Carcinogenicity of Di(2-ethylhexyl) Phthalate (DEHP) in Perinatal versus Adult Exposed HSD: Sprague-Dawley SD Rats

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Di(2-ethylhexyl) Phthalate (DEHP) is a plasticizer known to produce adverse effects on the developing male reproductive tract and on fertility via an anti-androgenic mechanism. Increased incidence and severity of male reproductive tract malformations occurs in rodents following exposure during critical developmental windows. DEHP is a known rodent carcinogen which induces hepatic, pancreatic, and testicular tumors in chronic studies. To date, no previous cancer assessments of DEHP have utilized a lifetime exposure paradigm that includes the perinatal and prepubertal periods. Currently, it is not clear if developmental exposure would alter lifetime DEHP-associated carcinogenic risk. To address this concern, the National Toxicology Program (NTP) evaluated if perinatal + lifetime DEHP exposure would impact the dose response, incidence and/or severity of the carcinogenic response in rodents relative to chronic exposure initiating in adulthood. Two cancer bioassays were conducted, in which 50 Sprague-Dawley rats per sex/group were exposed to 0, 300, 1000, 3000 or 10000 ppm of DEHP via dosed feed for 2 years, with differing exposure paradigms: adult only or perinatal + lifetime exposure. No effect on overall survival was attributed to DEHP in either study. Body weights of rats in the 300, 1000, and 3000 ppm groups were commensurate with controls in both studies. At study termination, lower body weights associated with decreased body weight gains throughout the study duration were observed in 10000 ppm groups in both perinatal (males -30%, females -32%) and adult (males -16%, females -22%) cancer bioassays, relative to controls. Identified tumor types in the liver and pancreas were consistent between studies. Higher incidences of pancreatic acinar and hepatocellular adenomas/carcinomas were noted in perinatally-exposed males and females, respectively, relative to adult-only exposures, although this finding was restricted to the 3000 ppm exposure groups. In contrast to previous reports, a higher incidence of testicular adenomas was observed in 10000 ppm adult-only exposures, relative to perinatally-exposed animals. In summary, these findings suggest that perinatal + lifetime exposure may alter the DEHP carcinogenic response in rodents relative to adult-only exposure.

1315 Alternative Splicing as a Key Mechanism in Arsenic-Induced Squamous Cell Carcinoma: Evidence from RNA-Seq and Proteomic Analysis

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Chronic exposure to arsenic, a group I carcinogen, leads to multi-organ cancers, skin being the primary target, although, the molecular etiology remains elusive. Arsenic exposure has been reported to induce differential mRNA splicing. Abrogation of normal alternative splicing patterns have been linked to several cancers, as the expression of many cancer-related genes are regulated by alternative splicing. We hypothesized that differential mRNA splicing in protein coding genes could be a driving force in an established cell line model of arsenic-induced skin carcinogenesis. HaCaT cells exposed to 0 (As^-) or 100 (As^+) nM $NaAsO_2$ were maintained for 7 weeks in independent quadruplicates. RNA and proteins were isolated from each sample. RNA-seq was carried out followed by analysis of the data by rMATS algorithm to identify significant differential splicing events between the As^- and As^+ samples. In addition, we performed ESI tandem mass spectrometric analysis to quantify differential proteome expression in whole cell lysates of the same samples. 1,149 skipped exon events were significantly different between As^- and As^+, of which 61.5% resulted in the protein coding changes. From proteomic analysis, 227 differentially expressed proteins were found. Gene ontology (GO) analysis on the alternatively spliced genes found enrichment in several splicing related pathways (83 unique genes). In accordance, GO analysis of the proteomic profile also showed splicing as a major enriched category (37 unique proteins). Comparison of over-represented pathways from alternative splicing and proteomic data revealed 3 common molecules: Splicing Factor SWAP (*SFSWAP*), DEAD-Box Helicase 42 (*DDX42*) and Serine/Arginine Repetitive Matrix 2 (*SRRM2*). These genes are related to mRNA splicing and are associated with cancer. Presence of overlapping unique genes/proteins in the two datasets that are involved in the same splicing regulation pathways suggests that global modulation of mRNA splicing by arsenic exposure plays a role in arsenic-induced carcinogenesis. *Funding: NIH grants R01ES027778, R21ES023627, P20GM103436, R15GM126446, P20GM113226, P30GM106396.*

1316 Bis-Indole Derived Nuclear Receptor 4A1 (NR4A1) Antagonists Inhibit TGF-β-Induced Invasion of Embryonal Rhabdomyosarcoma Cells

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Transforming growth factor β (TGFβ) enhances invasion of breast and lung cancer cells through phosphorylation-dependent nuclear export of the nuclear receptor 4A1 (NR4A1, Nur77). This response is inhibited by the NR4A1 antagonist 1,1-bis(3'-indolyl)-1-(phydroxyphenyl)methane (CDIM8) and we hypothesized that similar effects would be observed in Rhabdomyosarcoma (RMS) cells. Although some kinase inhibitors block TGFβ-induced invasion of embryonal RMS (ERMS) cells, the mechanism differs from breast and lung cancer cells since NR4A1 is extranuclear in ERMS cells. However, CDIM8 blocks basal and TGFβ-induced invasion of RD and SMS-CTR ERMS cell lines but not Rh30 alveolar rhabdomyosarcoma cells. Moreover, the response in ERMS cells was independent of SMAD7 degradation or activation of SMAD2/SMAD3. β-catenin silencing decreased ERMS cell invasion and CDIM8 induced proteasome-independent downregulation of β-catenin. The novel mechanism of CDIM8-mediated inhibition of basal and TGFβ-induced ERMS cell invasion was due to activation of the bcl-2-NR4A1 complex, mitochondrial disruption, induction of the tumor suppressor-like cytokine interleukin-24 which in turn downregulates β-catenin expression. Thus, the NR4A1 antagonist inhibits TGFβ-induced invasion of ERMS cells through initial targeting of cytosolic NR4A1.

1317 Assessment of the Carcinogenic Potential of Pretomanid in Transgenic Tg.rasH2 Mice

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The nitroimidazole pretomanid (PA-824) was recently approved by FDA for treatment of extensively drug resistant tuberculosis (XDR TB). Nitroaromatic compounds as a class carry a risk of being genotoxic and potentially carcinogenic based on reactive metabolite formation. Pretomanid was found to be nongenotoxic in a series of *in vitro* and *in vivo* studies (with and without liver S9 fraction), but a minor metabolite known as M50 was found to be positive in an Ames assay. To assess the *in vivo* carcinogenic potential of pretomanid, hemizygous Tg.rasH2 mice were administered the compound once daily by oral gavage administration for 26 weeks. Male mice were given pretomanid in vehicle at doses of 0, 5, 15 and 40 mg/kg/day. Female mice were given pretomanid in vehicle at doses of 0, 10, 30 and 80 mg/kg/day. Positive control mice of both sexes received intraperitoneal injections of urethane at 1000 mg/kg on Days 1, 3 and 5. There were no pretomanid-related early deaths, tumors, non-neoplastic microscopic findings, or gross necropsy findings at any dose level. The positive control gave the anticipated response. Oral administration of pretomanid to mice produced plasma exposure to both parent compound (high dose AUC was 2 to 3 times the clinical AUC at the maximum recommended human dose) and its M50 metabolite, with exposure to pretomanid more than 10 times the exposure to the M50 metabolite at all dose levels in both sexes. These data show that pretomanid was not carcinogenic in a transgenic mouse model.

1318 A Retrospective Analysis of Transgenic Mouse Carcinogenicity Studies for Pharmaceuticals in Japan

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Rat 2-year carcinogenicity studies of 182 compounds were retrospectively analyzed by PhRMA (*Toxicol. Pathol.* 39, 716-744, 2011). Taken together with retrospective analyses for the various datasets (PhRMA, FDA, JPMA, and EU + FDA), it has been hypothesized that based on evidences for pharmacology,

genotoxicity, and chronic toxicity the outcome of a 2-year rat carcinogenicity study can be predicted. In the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), S1 (carcinogenicity study) Expert Working Group proposed a prospective evaluation period to verify the possibility of waiving rat 2-year carcinogenicity studies by integrative analysis of Weight-of-Evidence (WOE) factors such as pharmacological action, genotoxicity, tumor-related lesions in rat 6-month toxicity studies and hormonal perturbation. Meanwhile, in the ICH S1B guideline a mouse study is also required to assess carcinogenic potential of a pharmaceutical in addition to the rat study. According to the current S1B guideline, medium-term transgenic (Tg) mouse carcinogenicity studies can be used instead of the long-term mouse studies. Depending on the ongoing ICH S1 prospective evaluation, the rat 2-year study can be waived. In such cases, the Tg mouse study will increase its weight. In this context, a comprehensive survey on Tg mouse carcinogenicity studies should be important in revising S1 guidelines. Here we retrospectively analyzed Tg mouse carcinogenicity studies (about 40 studies) for pharmaceuticals approved in Japan since 2007. The current S1B guideline has proposed several Tg mouse assays including p53+/- deficient, Tg.AC, Tg rasH2 and XPA deficient models. Recently, the use of Tg rasH2 mouse studies is readily increased to assess carcinogenic potential of pharmaceuticals. In this session, the implementation status of carcinogenicity studies using Tg mice, and the exposure margin of clinical dose with the highest dose and the tumor outcomes in carcinogenicity studies using rasH2 mice will be presented.

1319 Phenotypic Alterations and Cancer Progression in Human Uterine Leiomyoma Cells following Continuous Cadmium Exposure

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Cadmium (Cd) is a ubiquitous heavy metal found in the environment and poses serious health risks. Cd exposure has previously been linked to lung and prostate cancer. Cd has also been identified as a "metalloestrogen", or estrogen-mimic. In women with benign estrogen-responsive uterine fibroids, elevated blood Cd levels have been correlated with increased fibroid tumor growth. In this study, cadmium exposure was used *in vitro* to analyze if human uterine leiomyoma (ht-UtLM; fibroid) cells could be transformed into a malignant phenotype. Fibroid cells were exposed to 10 μM $CdCl_2$ for 8-weeks and analyzed by time-lapse confocal imaging, flow cytometry, transmission electron microscopy, Ki-67, and soft agar assays. Total RNA was extracted from fibroid cells for differential gene expression analysis using NanoString PanCancer Progression/Pathways Panels. We found that after 8 weeks of Cd exposure, a robust and fast-growing Cd Resistant Leiomyoma culture (CR-LM) was established. CR-LM cells showed changes in nuclear and cytoplasmic morphology and had significantly enhanced cell motility and cell proliferation compared to passage-matched ht-UtLM controls. CR-LM cells were able to form viable colonies in soft agar thus exhibiting anchorage-independent growth. NanoString analysis showed a significant downregulation of genes encoding for extracellular matrix (ECM) proteins; whereas matrix metalloproteinase (MMP) genes, involved in ECM degradation, were dramatically upregulated. A human MMP antibody array analysis confirmed increased protein expression of several MMPs in the CR-LM cells. Ingenuity Pathway Analysis (IPA) of the dataset predicted inhibition of the TGFB1 pathway network which could activate cancer cell proliferation. Overall, these data suggest that continuous Cd exposure induces progression towards a malignant phenotype in human fibroid cells *in vitro* and that chronic environmental cadmium exposures may pose a cancer risk for women with uterine fibroids.

1320 Cytotoxicity Assessment of Zinc Oxide Nanofibers in K562 Cells

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Zinc oxide (ZnO) nanofibers (NFs) are used for water and air cleaning as well as in cosmetic products such as sunscreen. This functionality can be attributed in part to their wide band gap energy and propensity for photocatalytic activity. They are also being considered for medical devices and novel drug delivery. Therefore, research is needed to examine the toxic effects from exposure to these nanofibers and their metal oxides. Previous studies have found exposure to cause reactive oxygen species (ROS)production which induced apoptosis and oxidative stress as well as cellular inflammatory response. The goal of this study was to examine the cytotoxic effect of ZnO NFs on K562 cells, a human myelogenous leukemia cell line. The ZnO nanofibers were synthesized from polyvinylpyrrolidone and zinc acetylacetonate hydrate in ethanol with the typical electrospinning technique. The fibers were then characterized with scanning electron microscopy (SEM), FT-IR, powder X-Ray diffraction, and Raman spectroscopy. The K562 cells were maintained and exposed to ZnO nanofibers at varied concentrations and time points. Cell proliferation, oxidative stress and inflammatory response were measured using MTT assay, LDH leakage assay, and quantitative real-time polymerase chain reaction (qRT-PCR). Upon ZnO NF exposure, K562 cells did not indicate significant cell death as evidenced by MTT and LDH leakage assay. Inflammatory response and oxidative stress were observed by gene expression. The most toxic concentration was 1 ug/mL. A possible explanation for the low cytotoxicity observed could be the non-adherent characteristic of K562 cells. This is due to a proposed mechanism that ZnO NFs attack the plasma membrane and then penetrate the cells.

1321 Locally Administered HB-EGF Reduces Radiation-Induced Oral Mucositis in Mice

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There is an unmet medical need for oral mucositis, which can occur with chemo radiation treatment of non-head and neck cancer. We investigate the efficacy of locally administered heparin-binding epidermal growth factor-like growth factor (HB-EGF) to the oral mucosa as a potential therapy to treat radiation induced oral mucositis. Using a single dose (20Gy) of X-ray irradiation to the heads of female C57BL/6J mice, we evaluated the efficacy of post irradiation treatment of HB-EGF (5 μl of 10 μg/ml) solution. The results shown that HB-EGF delivered post radiation significantly increase area of epithelial thickness and cell division of both tongue and buccal. This data provides the proof of concept that local administration of HB-EGF has the potential application to mitigate oral mucosal following radiation and shows promise for developing an effective local treatment.

1323 Investigating the Effects of Elevated Reactive Carbonyl Species Due to Glyoxalase 1 Knockdown and Inhibition in Cancer Cells

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The accumulation of reactive carbonyl species has been implicated in the pathology underlying diabetes, aging, and cancer. Glyoxalase 1 (Glo1) is over-expressed in tumor biopsies relative to adjacent tissue in breast and prostate cancer patients. Glo1 is part of a two enzyme, glutathione dependent detoxification system of the reactive carbonyl species, methylglyoxal (MG) and glyoxal. MG is a by-product of glycolysis that can form advanced glycation end-products (AGEs) and result in protein, RNA and DNA damage. MG accumulation can lead to DNA adducts that cause mutations and single strand breaks. The purpose of this study is to investigate the relationship between DJ-1, Nrf2, and the Glyoxalase 1/2 (Glo1/2) detoxification pathway. We knocked down Glo1 using siRNA in a panel of breast and prostate cancer cell lines. We also used the glutathione analogue, S-p-bromobenzylglutathione cyclopentyl diester ($pBrBzGSH(Cp)_2$) to inhibit Glo1. In cancer cells, DJ-1 is over expressed. DJ-1 has been found to have deglycase activity and may provide defense against reactive carbonyl species induced AGEs that are formed as a by-product of cellular metabolic activity such as glycolysis and lipid peroxidation. We have conducted western blots and quantified Glo1, Glo2, DJ-1, Nrf2 and beta-actin expression levels in the Glo1 knockdown (KD) and siRNA scrambled control cells. We did not observe any significant differences in DJ-1 expression in the Glo1 KD cells relative to the controls. We are currently investigating the relationship between Glo1 KD and Nrf2 stabilization and the elevation of members of the aldo-keto reductase (AKR) superfamily. Additionally, the upregulation of GSH due to the Keap1-Nrf2 pathway is being investigated by probing for two essential enzymes found in the GSH biosynthesis pathway, gamma-glutamylcysteine synthetase and glutathione synthetase. *This work was supported by a CSUPERB New Investigator Award and an NIH SCORE SC2 award to D.T.*

1324 Effects of Dietary Fish Oil and Omega-3 Fatty Acids on the Carcinogenic Polycyclic Aromatic Hydrocarbon (PAH)-DNA Adduct Levels in Mouse Lung *In Vivo*

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Many epidemiological studies and animal experiments have shown positive correlation between levels of carcinogen-DNA adducts and tumor incidence. Therefore, decrease in DNA adduct levels is expected to attenuate tumor formation. In this study we report that dietary fish oil (FO) and omega-3 fatty acids attenuate the formation of DNA adducts mediated by polycyclic aromatic hydrocarbons (PAHs). B6C3F1 male mice were pre-fed a FO or corn oil (CO) diet for 30 days, or A/J male mice were pre-treated with omega-3 fatty acids eicosapentaenoic acid (EPA, 50 mg/kg and 100 mg/kg) and/or docosahexaenoic acid (DHA, 30 mg/kg and 60 mg/kg) for 3 days. The B6C3F1 mice were then treated with a mixture of seven carcinogenic PAHs including benzo(a)pyrene (BaP) with two doses. A mixture of high dose PAHs mixture contained BaP, 1.72 µg; BA, 5.25 µg; chrysene, 5.25 µg; BbF, 2.15 µg; BkF, 1.33 µg; DBA, 0.11 µg and IP, 0.67 µg for 20 g body weight. These chemicals and their concentrations were similar to the residues extracted from contaminated soils of SuperFund sites. The low dose PAHs were proportionally reduced by 2.5 fold from high dose PAHs mixture. Animals were terminated at 1, 3, or 7d after treatment of PAHs or BaP. A/J mice were treated with pure BaP (25 mg/kg) via a single intraperitoneal injection. Animals were sacrificed 48 h after BP treatment. The levels of DNA adducts were analyzed by the ^{32}P-postlabeling assay. Our results showed that the levels of total PAH-DNA adducts in the lungs of B6C3F1 mice at 3 and 7 d after treatment of a mixture of PAHs were significantly decreased in FO compared with CO group. A/J mice pre-treated with FO, EPA and/or DHA also showed significant decreases of total pulmonary BaP-DNA adducts and/or BPDE-dG, compared to those in mice that did not receive the omega-3 fatty acids. Interestingly, low doses of EPA and DHA, given in combination, were more efficiently and significantly attenuated the levels of total pulmonary DNA adducts by 42% and levels of BPDE-dG by 45%. Furthermore, western blot analysis showed that EPA and DHA significantly inhibited the expression of CYP1B1 that plays a key role in the bio-activation of BP to DNA-binding metabolites. Reverse phase protein array (RPPA) analysis suggested a possible mechanistic link between detoxification of PAHs by omega-3 fatty acids and DNA adduct formation.

1325 Regulation of Basic Fibroblast Growth Factor (bFGF)-Induced Angiogenesis by the Small GTPase RhoA

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Tumor vessels display abnormal structure and function due to imbalanced angiogenesis. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the best known pro-angiogenic factors in this process. The goal of antiangiogenic therapy is to inhibit tumor growth by oxygen and nutrient deprivation. Current antiangiogenic therapies target VEGF/VEGFR signaling pathways. However development of resistance due to upregulation of bFGF remains a serious disadvantage of current approaches, leading to efforts of simultaneous targeting of both VEGF and FGF pathways. A small GTPase RhoA has been reported to regulate VEGF-induced angiogenesis. In the present study we investigate the role of endothelial RhoA in bFGF-induced angiogenesis to identify whether RhoA can be considered a target for both VEGF- and FGF-induced angiogenesis. We performed RhoA pulldown assays to identify RhoA activation downstream of bFGF treatment. We used Boyden chamber cell migration assay, 2D tube formation assay using matrigel and 3D sprouting assay with collagen to assess angiogenic potential. Endothelial RhoA was knocked down by siRNAs or blocked by pharmacological inhibitors. Biochemical assays were performed to identify the molecular mechanism of bFGF-induced RhoA activation. *In vivo*, endothelial-specific inducible RhoA-deficient mice with a fluorescent reporter were used in a newly modified matrigel plug angiogenesis model allowing identification of endothelial RhoA excision efficiency. bFGF treatment activates RhoA in human umbilical vein endothelial Cells (HUVEC). RhoA inhibition with C3 toxin or RhoA knockdown abrogated bFGF-induced endothelial cell migration and tube formation *in vitro*. *In vivo*, endothelial RhoA deficiency blocked bFGF-induced angiogenesis in matrigel plug assay. Among the bFGF receptors, FGFR1 is predominantly expressed in HUVECs and FGFR1 knockdown abrogated bFGF-induced RhoA activation and angiogenesis. RhoA inhibition partially blocked bFGF-induced JNK phosphorylation but did not affect ERK and p38 activation. Ongoing experiments are aiming to delineate the precise role of endothelial RhoA in the FGF downstream pathway. Collectively, our data suggest that RhoA pathway participates in bFGF-induced angiogenesis and can be a potential target for anti angiogenic therapy.

1326 Sulforaphane Inhibits Colon Adenoma Organoid Formation and Induces Differentiation in a Dose-Dependent Manner

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Colorectal cancer ranks as the 2nd leading cause of cancer mortality in the United States. Preventive strategies for individuals with familial colon cancer syndromes remain limited. Sulforaphane (SFN), an isothiocyanate in cruciferous vegetables and potent activator of the Nrf2 transcription factor, has chemopreventive effects *in vitro* and *in vivo*, including inducing growth arrest and/or apoptosis. We have previously demonstrated that SFN inhibits breast cancer stem cells, which are the hypothesized cells of origin for carcinogenesis responsible for cancer invasion and metastasis. Whether SFN can act as a cancer preventive agent through targeting colon stem cells is unknown. To investigate this, we treated APC-mutant patient-derived human colon adenoma organoids with 4 doses of SFN (1.5, 3.1, 6.25, and 12.5 µM) or DMSO-control for 6 days and quantified the number and size of organoids formed. Additionally, we performed RNAseq on each treatment group and calculated benchmark doses for pathway alterations using BMDExpress2. Organoid formation decreased with SFN treatment in a dose-dependent manner and the highest dose decreased organoid formation by 80% compared to control. The number of differentially expressed genes also increased in a dose-dependent manner. Organoids treated with the lowest SFN dose had 254 differentially expressed genes (83 down-regulated and 171 up-regulated) compared to control while 7099 genes (3478 down and 3621 up) were differentially expressed in the highest dose. Increasing doses of SFN increased transcription of many Nrf2 target genes, including *NQO1*, *HMOX1*, and *ALDH3A1*. Increasing doses of SFN also increased the expression of colon differentiation genes, including *KRT20*, *AQP3*, and *ALPP*, and decreased the expression of colon stem cell genes *LGR5*, *OLFM4*, and *EPHB2*. BMDExpress2 analysis estimated the median benchmark dose for the gene ontology "stem cell proliferation" pathway at less than 1µM. Ongoing work is characterizing the stem cell specific effects of SFN in a mouse model of colorectal carcinogenesis. These results provide evidence that SFN induces a differentiation-associated phenotype in colon adenoma cells at physiologically relevant doses.

1327 The Importance of the Aryl Hydrocarbon Receptor Signaling Pathway on Mammary Tumorigenesis

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The aryl hydrocarbon receptor (AhR) is known for mediating the toxicity of environmental pollutants such as dioxins and numerous dioxin-like compounds. Due to the widespread occurrence in our environment and the high toxic potential, these contaminants are of concern to promote breast cancer. Several studies have shown that the AhR is critically involved in mediating defects of the mammary gland differentiation and suppresses mammary gland development. More recently it has been found that the AhR may also act as a critical receptor protein in tumor promotion independent from exogenous ligands, which is based on its role in immune tolerance and increased survival in cancer cells. Consequently, the AhR may serve as an attractive target for new drugs in breast cancer therapy. AhR's action is restricted by a specific repressor protein, the AhR Repressor (AhRR). Interestingly, a recent report found that breast cancer patients who had low AhRR expression also had shorter metastasis-free survival and identified AhRR as an independent prognostic factor. While AhRR effectively blocks AhR, the role of AhRR as a tumor suppressor gene is only poorly understood. We hypothesize that AhRR will oppose AhR-mediated mammary tumorigenesis. Results: We established a transgenic mouse (AhRR Tg mice) that overexpresses AhRR and discovered that these mice were protected from tumor promoting effects induced by dioxin. To test the tumor-suppressive action of AhRR *in vivo*, we used a syngeneic murine tumor model in which E0771 mammary tumor cells are engrafted in the mammary fat pad. Results indicate a significantly suppressed tumor growth of E0771 cells in AhRR Tg mice compared to control B6 mice. Tumor growth was enhanced in dioxin-treated wt mice, as expected, but was significantly suppressed in AhRR Tg mice, supporting our hypothesis. Moving forward, we plan to examine the impact of AhRR overexpression in other mammary tumor models. The polyoma middle T antigen (PyMT) mouse model is a well-accepted model of ER-negative, metastatic breast cancer. Our data show, that the expression of AhR increases significantly in mammary tumors during progression in PyMT mice. In contrast, the expression of AhRR

decreases. Furthermore, expression of inflammatory markers (e.g. COX-2 and C/EBPβ) increases during tumor progression. The preliminary results indicate that the PyMT mouse is a suitable model to study the role of AhR and AhRR in breast tumorigenesis since it reflects a similar expression pattern found in human breast cancers. Our data indicate that AhRR restricts AhR-dependent expression of pro-inflammatory cytokines and acute toxicity. We also show that the AhRR represses the PKA-C/EBPβ inflammatory axis, induced by AhR activation, supporting its role as a tumor suppressor. Furthermore, AhRR opposes AhR-driven tumor cell survival. In summary the results show that AhRR suppresses mammary tumor cell growth *in vivo* and regulates genes involved in inflammation and apoptosis. The results will help us to understand AhRR's function as a tumor suppressor gene and the potential biomarkers of mammary tumorigenesis mediated by AhR and environmental toxicants.

1328 Drinking Water Bromate May Promote Carcinogenesis through Both Genotoxic and Nongenotoxic Mechanisms at Low Dosages

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Bromate is a by-product of currently implemented disinfectants for drinking water in many countries, including the US. While linear extrapolation of male rat tumor data has been used to set the Maximum Contaminant Level (MCL), this is largely based on the assumption that direct oxidation of DNA is the responsible carcinogenesis mechanism. However, immunohistochemical and mass spectroscopy methods previously found that bromate can brominate tyrosines *in vivo*. Our data likewise suggests that bromate can brominate proteins at tyrosines *in vitro* and *in vivo,* which in turn potentially activates well characterized carcinogenesis pathways. Furthermore, bromination appears to change gene expression across several tissues and modulates serum and cellular free iron concentrations by modifying transferrin. The change in free iron would be expected to increase oxidative stress and is an alternative mode of action to the formation of 8-oxoG adducts in DNA. As the increase in free iron begins at the lowest dose we administered to rats for 28 days (5 mg/L vs the previously observed 250 mg/L in drinking water required to increase 8-oxoG), our data suggest this mechanism is more likely to occur at low doses.

1329 Comparison of NOAEL, ETD_{10}, and $BMDL_{10}$ Values for Nongenotoxic Carcinogens: Which Point of Departure Is Most Sensitive?

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Human carcinogens may act by different modes of action, but generally can be classified as genotoxic and non-genotoxic carcinogens. The aim of this study was to better understand which endpoints and/ or point of departures are the most sensitive after chronic oral exposure for non-genotoxic carcinogens. 232 compounds which were classified as non-genotoxic carcinogens, using a simple decision tree, were identified. Following a detailed review of all available peer-reviewed literature, experimental and predicted data, this dataset was reduced to 223 organic compounds. NOAEL values were derived for these 223 compounds from chronic and (if required) subchronic studies following oral application using well established high quality databases e.g. RepDose, ToxRefDB, COSMOS or peer-reviewed publications. As far as possible, NOAEL values were derived from the same study as used in the CPDB database for the calculation of carcinogenic potency (TD_{50} values). NOAELs were based on either the most sensitive i) adverse apical effect in the entire study; ii) non-neoplastic lesion; or iii) neoplastic lesion. Study quality was considered as one potential confounder. These NOAELs are compared to the effective tumour dose (ETD_{10}) and the benchmark dose level ($BMDL_{10}$) calculated by model averaging, where a tumor-related effect is expected to be observed in 10% of the animals tested. The comparative analysis of the correlations between NOAEL/EDT/BMDL values revealed that compounds with a concern for bioaccumulation were found among the 5% most toxic compounds. After exclusion of these compounds, the 5th percentile of the chronic NOAELs is in the same range as of $BMDL_{10}$ values, whereas the 5th percentile of the EDT_{10} is about 3 times higher than the NOAEL. These results were evaluated with regard to the current threshold of toxicological concern (TTC) threshold. *This work received funding from the CEFIC LRI B18_2 project.*

1330 Differential Cytotoxicity of Doxorubicin in Renal Cancer Cells Is Independent of VHL and p53 Status

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Differential sensitivity of cancer to chemotherapeutic drugs is a growing concern worldwide. Although not yet clearly known, the primary basis of differential sensitivity is believed to be governed by the genetic/epigenetic makeup of the tissue bearing cancerous growth. The objective of this study was to understand the role of the genetic background of renal cancer cells on the sensitivity to chemotherapeutic drug doxorubicin. Two renal cell carcinoma cell lines Caki-1 and 786-0 with different status of tumor suppressor genes *p53* and *VHL* were used in this study. Sub-lethal concentrations of doxorubicin were used for the treatment of cells. The cytotoxicity of doxorubicin was determined by MTT cell viability assay. The effect of doxorubicin on the cell cycle was evaluated by flow cytometry. The expression of tumor suppressor genes *VHL* and p*53, as well as* the apoptosis-related genes Bax and Bcl2, were measured by quantitative real-time PCR. The MTT data revealed that 786-0 cells were more sensitive to doxorubicin as compared to Caki-1cells. Cell cycle analysis further revealed that doxorubicin caused more G2/M arrest in 786-0 cells than in Caki-1 cells. However, the expression of p53 and VHL was much greater in doxorubicin-treated Caki-1 cells than 786-0 cells. The findings of this study suggest that chemotherapeutic drug doxorubicin is more cytotoxic to primary renal cancer 786-0 cells with mutant VHL and p53 than the metastatic Caki-1 cells with wild type VHL and p53. More importantly, our data suggest that doxorubicin-induced cytotoxicity in these two renal cancer cell lines are independent of p53 expression level.

1331 Endoplasmic Reticulum Stress and P38 Signaling Pathway Contribute to the Cytotoxicity of Perhexiline in Hepatic Cell Lines

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Perhexiline is a prophylactic antianginal agent developed in the 1970s and used worldwide. Despite its therapeutic success, its use diminished due to reports of severe side effects including hepatotoxicity in certain patients. The mechanisms underlying, however, is unclear yet. Here using HepG2 cells and HepaRG cells, we characterized the molecular mechanisms in the cytotoxicity of perhexiline. We found that perhexiline induced endoplasmic reticulum (ER) stress in HepG2 cells. Exposing HepG2 cells to perhexiline increased the gene expression level of classic ER stress markers including PERK, IRE1α, ATF6, ATF4, and CHOP. The protein levels of CHOP, ATF4, and peIF2α were also elevated. Using a *Gaussia* luciferase reporter assay, perhexiline-suppressed protein secretion was evaluated, and defects in ER function was confirmed. Pretreatment with ER stress inhibitor 4-PBA, or knockdown of ATF-4 gene attenuated the perhexiline-induced ER stress and cytotoxicity. In addition, perhexiline activated all three branches of MAPK signaling cascade, as evidenced by increased phosphorylation of P38, JNK, and ERK1/2. Inhibition of P38 by inhibitor SB239063 significantly reduced the perhexiline-induced cytotoxicity and ER stress. Taken together, our findings suggest that ER stress and the P38 signaling pathway contributes to the cytotoxicity of perhexiline in hepatic cell lines.

1332 Novel Drug Targets for Inhibiting Tumor Growth and Immunotherapy-Related Checkpoints

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Specificity protein transcription factors (Sp1, Sp3 and, Sp4) and members of the orphan nuclear receptor 4A subfamily (NR4A1, NR4A2 and, NR4A3) are highly expressed in most solid tumors and their derived cell lines compared to corresponding non-tumor tissues. Several studies in our laboratory have shown that many anticancer agents including ROS-inducers downregulate expression of Sp TFs in multiple cancer cell lines. We have also shown that NR4A1 acts as a cofactor for Sp1 or Sp4-mediated gene expression and CDIM/NR4A1 antagonists inhibited these responses in various cancer cell lines. The highly selective killing of cancer cell lines by piperlongumine is due to ROS-dependent epigenetic repression of c-Myc, which leads to downregulation of miRs 17, 20a, 27a, upregulation of ZBTB 4 and ZBTB10 which are Sp repressors and downregulate expression of Sp1, Sp3, Sp4, and pro-oncogenic Sp-regulated gene products. Sp TFs were also shown to be vital for the growth of multiple myeloma (MM) cells and similar results were observed after treatment of MM cells with bortezomib which is clinically used for treating this dis-

ease. Subsequent studies indicate that the underlying mechanism of action of bortezomib in MM cells is due to caspase-8 dependent downregulation of Sp TFs. We also investigated the role of NR4A2 in glioblastoma cells and showed that knockdown of NR4A2 using antisense oligonucleotides inhibited growth, induced Annexin-V staining (apoptosis) and inhibited migration/invasion. Bis-indole derived NR4A2 ligands (C-DIMs) mimicked the functional responses observed in glioblastoma cells after NR4A2 knockdown indicating that NR4A2 ligands acts as NR4A2 antagonists and these compounds represent a novel approach for treating GBM. NR4A1 is overexpressed in both ER-positive and ER-negative breast cancers and high expression of NR4A1 predicts decreased patient survival and we demonstrated that NR4A1/Sp1 regulates the PD-L1 checkpoint ligand in TNBC cells and it can be decreased after treatment with CDIM/NR4A1 antagonists and enhance tumor immunity. Cl-OCH3, a buttressed CDIM 8 analog which acts as an immunotherapy mimic inhibited mammary tumor growth and decrease lung metastasis and increased T_{eff} to T_{reg} ratio compared to untreated mice. Thus CDIM/NR4A1 antagonist are novel small molecule immunotherapy mimics and checkpoint inhibitors that are being developed for clinical applications.

1333 Mitigating Toxicity through Targeted Therapy for SETBP1-Mutant Leukemia

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Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative neoplasm characterized by the overproduction of neutrophils. Historically, therapeutic options for CNL were limited to stem cell transplantation or hydroxyurea, a cytotoxic agent with a low therapeutic index. The discovery of CSF3R mutations in CNL represented the first opportunity for targeted therapy. CSF3R mutations lead to ligand-independent receptor dimerization and JAK pathway activation. Ruxolitinib, a JAK inhibitor, is currently in clinical trials for CNL. However, while ruxolitinib remains a promising therapy, some patients develop co-occurring mutations and relapse. One of the genes that is frequently mutated with CSF3R is SETBP1, which mutated in 45% of CNL patients. SETBP1 mutations predict poor overall patient survival and the mechanisms of SETBP1 in disease progression and relapse are poorly understood. In order to generate a preclinical disease model to evaluate the role of SETBP1, we made a novel hematopoietic cell line co-expressing mutant CSF3R and SETBP1. When this cell line was injected into C57BL/6 mice, myeloid leukemia developed in four weeks. A second murine model was developed by transplanting primary bone marrow transduced with CSF3R/SETBP1 into Balb/c mice, which resulted in neutrophilic leukemia in 17-19 days. Finally, doxycycline-inducible cell lines were generated to study the effect of withdrawing SETBP1 or CSF3R. To identify novel therapeutic targets, we conducted a small molecule screen on our CSF3R/SETBP1 cell line with 172 inhibitors of cancer-associated pathways. Top hits were evaluated for synergy with ruxolitinib. An irreversible inhibitor of lysine-specific demethylase 1 (LSD1) identified in this screen, and targets of LSD1 were confirmed to be upregulated by SETBP1 through RNAseq profiling of our doxycycline-inducible cell lines. To assess the tolerability of the LSD1 inhibitor and ruxolitinib, we treated healthy Balb/c mice with the LSD1 inhibitor (1.5 mg/kg/day) and ruxolitinib (90 mg/kg/day) by twice-daily oral gavage for two weeks (n=5/group). The drug combination resulted in weight loss but did not produce any hematologic abnormalities (white blood cells, hemoglobin, platelets). The combination will be evaluated for efficacy and tolerability in our murine leukemia models. In summary, we developed novel preclinical models that were used to identify a new, well-tolerated therapeutic strategy for SETBP1-mutant disease. In addition to therapeutic development, these models can be utilized in future studies of gene-environment interactions, and to evaluate the role of occupational and environmental exposures in leukemia progression.

1334 Cadmium Metal Exposures as a Driver of Gallbladder Epithelial Signaling Dysfunction and Chronic Inflammation in Gallbladder Cancers

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Gallbladder carcinoma (GBC) is a deadly malignancy in the human gastrointestinal system (5 year survival - 3-8%). Native Americans (NA) living in New Mexico (NM) have a 5-8 fold higher incidence of GBC compared to Caucasians living in the state. We hypothesize heavy metal exposures may be responsible for the GBC incidence disparities seen here in NM and globally. Recent epidemiological data appears to support our key hypothesis. To understand GBC disparities in NM, we established a cohort of GBC patient samples and used next generation sequencing (DNA-seq) approaches to identify major driver mutational patterns. We also used primary human GB epithelial cell lines to measure effects of Cd exposure on GB cell signaling dysfunction. In particular, we focused on effects on Akt, MAPK and COX-2 signaling pathways. DNA-seq analysis results show the mutational patterns of GBC samples in NM for the first time. We observed an elevated incidence of missense mutations in the genes of Akt and MAPK pathways similar to findings in GBC patients from China. Of importance, we observed approximately 25% of NM GBC patients have a pharmacologically targetable HER2 amplification (n=6/24). In our GB epithelial cell studies, we observed a dose dependent reduction of cell viability of gallbladder epithelial cells following 24-hour Cd exposures. The measured EC50 values of gallbladder primary cells range from 11-18 μM. Importantly, we observe activation of the Akt and MAPK signaling pathways via increased phosphorylated activation. The ROS activity in GB epithelial cells is elevated due to Cd exposures with a reduction in the GSH levels. N-acetyl cysteine supplementation, in contrast, reduced the activation of Akt and MAPK signaling proving the role of Cd-induced oxidative stress in signaling disruption. A second key observation was the increased COX-2 gene expression via activation of the Akt and NF-kB pathways. Thus, our basic studies in aggregate prove two key defining and necessary features of gallbladder carcinogenesis: Akt signaling activation and chronic inflammation. Our initial studies demonstrate a role for environmental heavy metal exposures (Cd) in gallbladder carcinogenesis for the first time. Future research will seek to strengthen the role of Cd and other heavy metal exposures as drivers of gallbladder cancer disparities seen among Native American populations of NM.

1335 14-Day Nose-Only Inhalation Toxicity and Haber's Rule Study of NNK in Rats

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4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the key tobacco-specific nitrosamines which play an important role in humans lung carcinogenesis. However, repeated inhalation toxicity study data of NNK currently is limited. In the present study, subacute inhalation toxicity of NNK was evaluated in Sprague Dawley rats, both sexes (9-10 weeks age) exposed by nose-only inhalation of NNK in the following groups (16 rats/sex/group): air and vehicle controls, 0.8, 3.2, 12.5, and 50 mg/kg body weight (BW)/day of NNK (NNK aerosol concentration: 0, 0, 0.03, 0.11, 0.41, and 1.65 mg/L air) for 1 hour/day for 14 consecutive days. Whether the subacute inhalation toxicity of NNK follows Haber's Rule was also evaluated by exposure for additional three dose levels of 3.2, 12.5, and 50 mg/kg BW/day of NNK (NNK aerosol concentrations: 0.03, 0.11, and 0.41 mg/L air) for 4 hours/day for 14 consecutive days (7 rats/sex/group). Toxic responses were compared between the 4-hour exposure groups and the 1-hour exposure groups with the same daily exposure doses. Clinical observations, body weights, food consumption, clinical pathology, organ weights, and histopathological findings were compared with those of vehicle control. The micronucleus assay was conducted in peripheral blood and bone marrow. The results showed that there were multiple significant adverse effects in both sexes induced by NNK inhalation but no neoplasms or NNK-related deaths. NNK exposure did not increase micronucleus frequencies in the peripheral blood or bone marrow erythrocytes. The no observed adverse effect level (NOAEL) was 0.79 (male) and 0.87 (female) mg/kg BW/day or 0.03 mg/L air for 1 hour/day for both sexes, the lowest observed adverse effect level (LOAEL) was 2.96 (male) and 3.29 (female) mg/kg BW/day or 0.10 mg/L air for 1 hour/day for both sexes. The study component designed to test Haber's Rule indicated that 14-day inhalation exposure to the same dose at a lower concentration of NNK aerosol for a longer time (4 hours daily) caused greater adverse effects than exposure to a higher concentration of NNK aerosol for a shorter time (1 hour daily).

1336 KRAS-Retroviral Fusion Transcripts and Gene Amplification in Arsenic-Transformed, Human Prostate CAsE-PE Cancer Cells

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CAsE-PE cells are an arsenic-transformed, human prostate epithelial line containing oncogenic mutations in KRAS. In this *in vitro* transformation model, arsenic exposure produced a highly overexpressed, mutated KRAS allele in CAsE-PE cells versus the immortalized parent cells, RWPE-1, expressing normal KRAS transcript. We previously observed increased KRAS copy number in CAsE-PE cells, supporting a process of gene amplification of unclear origin. Here, we characterized the flanking genomic and transcriptomic sequences to KRAS in CAsE-PE cells for insight into KRAS amplification. Initial comparison

of DNA-Seq and RNA-Seq reads showed coincident spikes of reads aligned to all KRAS exons in CAsE-PE cells but in RWPE-1 cells RNA-Seq showed low KRAS exon reads and an even distribution of KRAS genomic reads. We then searched for KRAS fusions in DNA and RNA sequencing data, finding some reads aligning to KRAS and viral sequences. After generating cDNA, we designed short and long probes for hybridization and then sequenced cDNA fragments by PacBio. More KRAS reads were captured from CAsE-PE cDNA compared to RWPE-1 by each probe set, and only CAsE-PE cDNA showed presence of KRAS viral fusion transcripts, primarily mapping to LTR (long terminal repeats) and endogenous retrovirus sequences on either the 5′- or 3′-end of KRAS. A majority of KRAS viral fusion transcripts contained 4 to 6 exons but some PacBio sequences showed KRAS in unusual orientations, suggesting viral insertions occurred within the gene body. In other experiments, conditioned media were extracted for retroviral particles and RNA-Seq was performed on the isolates showing KRAS retroviral fusion transcripts in CAsE-PE media only. Truncated KRAS transcripts suggest multiple retroviral integration sites may occur within the KRAS gene, producing KRAS retroviral fusions of various lengths. The CAsE-PE *in vitro* arsenic-transformation model suggests prime molecular events in arsenic-derived tumors could include an arsenic mutation signature, production of driver oncogenes such as KRAS, activation of endogenous retroviruses with potential for genomic integration, and the formation of retroviral fusion transcripts for gene amplification. These findings suggest new avenues for future research in arsenic carcinogenesis.

1337 LC-MS/MS Assay Development Quantifying Mouse Plasma DFMO Concentrations

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Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, is being investigated for use in cancers. However, effective and safe dosing requirements based on pharmacokinetic modelling studies are needed. Accordingly, a quantitative assay using a Shimadzu triple quad LC-MS/MS, an Atlantis HILIC Silica 3µm 2.1 x 100mm column, and an isocratic mobile phase (75:25 acetonitrile and 0.2% formic acid) at a flow rate of 0.5mL/min was developed. Multiple Reaction Monitoring (MRM) quantified the precursor ion, 183m/z, using the following daughter ions: 183>120.10, 183>166.10, 183>80.05. Plasma standards and quality controls (QC) (20 uL) were processed using protein precipitation with cold acetonitrile. The lower limit of detection (LLOD) was 5ng/mL in neat solutions. Assay performance was determined from multiple runs (n=10) with standards from 250 - 50,000 ng/L and three levels of QC (500, 7500, and 40,000 ng/mL). Standard curves were linear with R2 values between 0.9960 and 0.9999. The assay was accurate and precise with a mean bias of <4% and RSD <0.5%. The assay was successfully applied to pharmacokinetic samples obtained from a DFMO pharmacokinetic (PK) study.

1338 ΔNp63α Suppresses Cell Invasion by Modulating Rac1 Activity

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ΔNp63α, a member of the p53 family of transcription factors, is overexpressed in a number of cancers and plays a role in proliferation, differentiation, migration and invasion. ΔNp63α has been shown to regulate several microRNAs that are involved in development and cancer. We identified miRNA miR-320a as a positively regulated target of ΔNp63α. Previous studies have shown that miR-320a is downregulated in colorectal cancer and targets the small GTPase Rac1, leading to a reduction in non-canonical WNT signaling and epithelial mesenchymal transition (EMT), thereby inhibiting tumor metastasis and invasion. We showed that miR-320a is a direct target of ΔNp63α. Knockdown of ΔNp63α in HaCaT and A431 cells downregulates miR-320a levels and leads to a corresponding elevation in PKCγ transcript and protein levels. Rac1 phosphorylation at Ser71 was increased in the absence of ΔNp63α, whereas overexpression of ΔNp63α reversed S71 phosphorylation of Rac1. Moreover, increased PKCγ levels, Rac1 phosphorylation and cell invasion observed upon knockdown of ΔNp63α was reversed by either overexpressing miR-320a mimic or Rac1 silencing. Finally, silencing PKCγ or treatment with the PKC inhibitor Gö6976 reversed increased Rac1 phosphorylation and cell invasion observed upon silencing ΔNp63α. Taken together, our data suggest that ΔNp63α positively regulates miR-320a, thereby inhibiting PKCγ expression, Rac1 phosphorylation, and cancer invasion.

1339 Why Do Whales Have Lower Cancer Rates? Whale Cells Avoid Particulate Chromate-Induced Homologous Recombination Repair Inhibition

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Whales are long lived species living in a complex environment that puts them at risk for long-term exposure and accumulation of environmental contaminants. However, they have low cancer rates, which may be due to better DNA protective mechanisms. How whales evade carcinogenesis is unknown. Particulate hexavalent chromium (Cr(VI)) is a human lung carcinogen. Cr(VI) induces DNA double strand breaks in human cells. Homologous recombination (HR) repair protects against these breaks by repairing them and preventing them from causing genomic instability and carcinogenesis. Prolonged particulate Cr(VI) induces DNA double strand breaks and suppresses HR repair in human lung cells. Whales are exposed to high Cr levels. This study focuses on the effect of particulate Cr(VI) exposure in whale lung cells to determine if they exhibit protective mechanisms against Cr(VI)-induced chromosome instability. Our study show Cr(VI) induced a concentration-dependent increase in DNA double strand breaks after both acute (24 h) and prolonged (120 h) exposure in whale lung cells, but did not inhibit HR repair in whale lung cells. Acute (24 h) Cr(VI) exposure caused G2/M arrest in human lung cells, but G1 arrest in whale lung cells. Prolonged (120 h) Cr(VI) exposure causes G2/M arrest in both human lung cells and whale lung cells. Future investigation of the differences in how human and whale cells respond to chemical carcinogens may provide valuable insight into mechanisms of preventing chemical carcinogenesis. *The work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].*

1340 A Scoping Review of Environmental Factors Affecting Breast Cancer Risk

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Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths in women worldwide. Although a genetic element exists, many breast cancer cases can be attributed to normal aging processes or environmental factors. Unlike age, environmental factors have the potential to be modified or avoided, and identification or characterization of these factors may aid in establishing breast cancer prevention priorities. This scoping review is being conducted to identify environmental factors that may influence breast cancer risk by categorizing published literature from humans, animals, and advanced cell models. The research collected in this review includes breast cancer and related non-cancer outcomes that indirectly alter breast cancer risk by impacting pathways related to breast and mammary gland development. PubMed was searched using a targeted search strategy to identify all relevant evidence on the mammary effects of toxicant exposure published between January 2008 and December 2018. The search strategy utilized mammary search terms to encompass breast structure, breast characteristics and processes, breast conditions and diseases, and lactation. Non-mammary sexual developmental indicator terms were also included. From the search we identified 1149 human studies, 559 animal studies, 64 *in vitro* studies involving 3D cultures or mammospheres, and 3 *in silico* studies. The studies were systematically categorized by key concepts of exposure (four domains and nine subdomains), evidence stream (human, animal, *in vitro*) and outcomes (cancer-related, breast and mammary gland developmental endpoints, and non-mammary-related) to develop an interactive evidence map. A recent update to the search retrieved 1297 articles that are currently being screened and categorized. The evidence map will provide an interactive method for individual researchers to search, sort, filter, and query published data linking environmental exposures and outcomes related to breast cancer risk. These data can also be used to develop a database of environmental risk factors potentially linked to breast cancer and provide a state of the science to inform future research decisions and systematic review themes.

1341 Estimating Acceptable Exposure Levels for Polybrominated Diphenyl Ether (PBDE) Based on *In Vitro* Models of Human Neurodevelopment and Biological Modeling

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Current acceptable exposure levels are mostly based on animal models, which are costly, time-consuming and may poorly predict adverse outcomes in humans. There is a need for alternative testing methods that are faster, cheaper, and provide human-relevant information. Our objective was to evaluate a method using human *in vitro* data and biological modeling to calculate an acceptable exposure level through a case study on PBDE developmental neurotoxicity. Using data from a study on human neuroprogenitor cells, we derived a point of departure using benchmark dose modeling for BDE-99 induced alteration of differentiation. We calculated a lower bound for the benchmark dose (BMDL) of 0.0832 µM for a benchmark response of 10%. We subsequently translated this BMDL expressed in terms of nominal concentration into a cellular level (2819 µg/kg cells) using the empirically-derived enrichment factor (60). We estimated the acceptable maternal daily intake (105 ng/kg/d) and plasma concentration (150 ng/g lipids) associated with a concentration of 2819 µg/kg in the child brain through reverse dosimetry using a pharmacokinetic model of gestational and lactational exposure. Finally, we compared the estimated maternal plasma BDE-99 level during pregnancy to median and maximum levels measured in epidemiological studies reporting associations with child neurodevelopment. Studies reported median levels ranging from <3.3 to 4.5 ng/g lipids, and maximum levels ranging from 169 to 298 ng/g lipids. Overall, the acceptable exposure level derived from *in vitro* data was higher than median levels measured in epidemiological studies, but in the range of maximum levels. Results suggest that factors related to the duration of exposure and interindividual variability may need to be taken into account.

1342 A Systematic Analysis of Mitochondrial Perturbation in Chemical-Induced Organ Toxicity Using High Content Imaging and High Throughput Transcriptomic Approaches

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Mitochondrial perturbation has been recognised as a key event in the process of chemical-induced organ toxicity. Nowadays the assessment of the mitochondrial functioning in early toxicity screens is based on static measurements of various mitochondrial processes. Here we focused on the integration of concentration- and time-resolved into an AOP framework to improve biological and mechanistic understanding of the relationship between mitochondrial perturbation and toxicity. We used a panel of >20 agrochemicals that specifically target mitochondrial respiratory complex (MRC) I, II or III and assessed both mitochondrial injury and subsequent cellular perturbations using high throughput imaging and transcriptomic platforms. We assembled a mitochondrial toxicity screening platform in HepG2 assessing the effects of MRC inhibitors on possible key events in a mitochondrial-related liver toxicity AOP. The various CI and CIII inhibitors, but not CII inhibitors, were effective in depleting complex inhibition, oxygen consumption and mitochondrial membrane potential, but not viability. Differentiated 3D HepG2 spheroids improved the prediction of complex inhibitor adverse effects. To quantify the relationship between mitochondrial perturbation and cellular signalling, we used TempO-Seq targeted RNA-seq assessing >3000 genes that capture the genome wide variations in gene expression upon MRC inhibitor exposure. The TempO-Seq data revealed gene expression upregulation that typically paralleled the potency and onset of mitochondrial toxicity by the various MRC inhibitors. Furthermore, using pathway analysis and gene clustering we identified individual genes and gene clusters could improve prediction of mitochondrial toxicity and provide the mechanistic insights on the cellular adaptation upon specific MRC complex inhibition. In summary, the integrated AOP based assessment of the dynamics of mitochondrial dysfunction and cellular stress response activation provides a novel mechanism-based approach to quantitatively in-depth assess mitotoxicity liability and discriminate the effect on different MRC complexes. *This work was part of the EU-ToxRisk project and received funding from the EU's Horizon 2020 programme under grant agreement No 681002.*

1343 Diisononyl Phthalate Exposure Affects Colonic Health in Adult Female Mice

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Diisononyl phthalate (DiNP) is a common phthalate that is used to make polyvinyl chloride flexible. DiNP is used in many end products such as toys, faux leather, and building materials. Exposure to DiNP has been shown to aggravate immune responses in humans. However, little is known about DiNP and its effects on the gastrointestinal tract. Thus, this study tested the hypothesis that subchronic exposure to DiNP alters colon histology and gene expression related to inflammation and cell cycle regulation. To test this hypothesis, 39-40 day old female CD-1 mice were orally dosed with corn oil vehicle (n=6), 20 µg/kg/day (n=6), or 200 µg/kg/day (n=6) DiNP for 10-14 days and then euthanized during diestrous immediately after dosing. Distal colons were collected for histological examination and gene expression analyses of *Ifng*, *Tnf*, *Il22*, *Il10*, *Bcl2*, *Ccnd2*, *Cdkn1a*, *Ccnb1*, *Bcl2l10*, *Ccne1*, *Cdk4*, *Ki67*, and *Pcna*. Histological analysis showed that DiNP exposure at 20 and 200 µg/kg/day increased colonic damage compared to control ($p=0.02$ and $p\leq0.05$, respectively). Colonic damage was mainly attributed to cellular infiltration and edema. However, enterocyte sloughing and aberrant colon walls were also observed in the DiNP treatment groups. Mice exposed to DiNP showed no significant differences in cytokine gene expression (*Ifng*, *Tnf*, *Il22*, *Il10*) in the colon compared to control. However, DiNP exposure increased *Ifng* and *Tnf* expression in a dose-dependent manner compared to control. Further, 200 µg/kg/day DiNP significantly downregulated *Ccnd2* compared to control. High DiNP did not alter the expression of *Bcl2*, *Cdkn1a*, *Ccnb1*, *Bcl2l10*, *Ccne1*, *Cdk4*, and *Pcna* compared to control. These data suggest that DiNP exposure causes colonic damage and may interfere with cell growth in the colon. *Supported by NIH T32 ES 007326, NIH R01 ES028661, and Vision 20/20.*

1344 Clotrimazole, a Potent PXR Agonist, Perturbs the Physiology and Metabolic Activity of the Gut Microbiome

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The Pregnane X Receptor (PXR) is a master regulator of xenobiotic metabolism in mammals, controlling the expression of genes including CYP3A4, sulfotransferases, and multi-drug transporters. The large hydrophobic ligand-binding domain of PXR allows it to respond to a broad repertoire of chemicals, with a variety of pharmaceuticals, herbal extracts, steroids, environmental toxicants, and several microbial metabolites (e.g., LCA) acting as its ligands. With a growing understanding of how xenobiotics, host metabolism, and the gut microbiome interact, it is important to determine the impact that PXR ligands have on the health and metabolism of the gut microbiome. The anti-fungal clotrimazole is a PXR agonist but also has known antibacterial activity. Using flow cytometry, we have shown that clotrimazole attenuates the membrane physiology (permeability and polarization) and biochemical activity of the collective cecal microbiome of mice, in a dose-dependent manner. Furthermore, clotrimazole significantly alters the metabolic profile of cecal microbes *in vitro*, as observed using untargeted LC-MS. Future work will aim to classify the microbes affected and understand the impact this might have on the host detoxification of additional PXR ligands. Since an intact and viable gut microbiome is essential for host health and regulation of xenobiotic metabolism, an understanding of how this relationship may be perturbed is of utmost importance.

1347 Establishment and Characterization of Rat Duodenal Organoids

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There is a need for additional nonclinical gastrointestinal models that can accurately model human physiology, including drug metabolism and toxicologic responses. For this reason, we have developed a rat duodenal organoid culture system that can more accurately represent the molecular and physiological features of the innate gastrointestinal environment. We isolated rat adult intestinal stem cells from whole rat duodenum and optimized culture conditions to stimulate growth and differentiation of organoids. Transmission electron microscopy (TEM), immunohistochemistry, mRNA and microarrays were used to characterize the organoids during differentiation. TEM was used to assess ultrastructural differences (such as apical microvilli, cytoplasmic vacuoles, perinuclear granules, adherens, tight junctions and desmosomes).

We stained the organoids with the following differentiation and intestinal region-specific antibodies by immunohistochemistry: zonula occludens-1 (ZO-1) for tight junction formation, mucin 2 for goblet cells, chromogranin A for enteroendocrine cells, and villin for enterocytes. Relative expression of lgr5 (adult stem cell marker), sucrose isomaltase (SI), villin and cholecystokinin (adult intestinal tissue markers) was measured by RT-PCR. As expected, lgr5 levels were down-regulated as SI, villin and cholecystokinin levels were up-regulated through the organoid differentiation period. Additionally, we used probe substrates for typical phase 1 and phase 2 drug metabolizing enzymes to assess organoid metabolism ability. Significant differences on metabolism between undifferentiated and differentiated organoids were observed. We aim to develop organoid structures from different segments of the intestine from human and preclinical species to provide a platform to investigate drug absorption and metabolism, intestinal physiology and disease, host-pathogen interactions and other toxicological questions.

1348 Discrepant *In Vitro* Cytotoxicity Results Can Be Explained by Method-Specific Differential Dependency on Pentose Phosphate Pathway-Associated Metabolism

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Tetrazolium reduction and resazurin assays remain the mainstay of routine *in vitro* toxicity batteries. However, neglecting to verify the baseline assumption of interaction of test article with method employed can produce potentially erroneous characterization of cytotoxicity/cell proliferation for test articles. The current investigation aimed to demonstrate the results of several standard cytotoxicity and proliferation assays varies in dependence on contributions from the pentose phosphate pathway (PPP). Beas-2B cells were treated with graded concentrations of benzo[a]pyrene (B[α]P) for 24 and 48 hours prior to cytotoxicity/proliferation assessment with commonly used MTT, MTS, WST-1, and Alamar Blue assays. B[α]P caused increased transient enhancement of metabolism of each dye assessed. An inhibitor of the glucose-6-phosphate dehydrogenase, 6-aminonicotinamide (6-AN), dose-dependently reduced metabolism of each endpoint listed in order of magnitude: WST-1 > MTS > MTT > Alamar Blue without overt cytotoxicity, implicating differential sensitivity of each endpoint to the pentose phosphate pathway (PPP). Nanomolar doses of B[α]P caused increases in all endpoints to suggest an increased cell proliferation. However, B[α]P treatment increased phosphorylation of Chk-1(S345) and p-53(S15), suggesting reductions in proliferative capacity. Reduction in proliferation was confirmed upon assessment of cell doubling time, which was lengthened dose-dependently. These results demonstrate differential sensitivity of studied cytotoxicity assessments on the PPP, thus 1) decoupling "mitochondrial activity" as an interpretation of cellular formazan and Alamar Blue metabolism, and 2) demonstrating the implicit requirement for investigators to sufficiently confirm methods of cytotoxicity/proliferation in routine experimentation. The nuances of these method-specific extramitochondrial metabolism must be scrutinized to properly qualify specific endpoints employed.

1349 Novel ID3 Regulatory Gene Networks Contributing to Brain Vascular Disease

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Cerebrovascular disease (CBVD) often leads to cognitive impairment and is a prominent comorbidity of Alzheimer's disease (AD) observed in 60%-90% of patients. Single nucleotide polymorphism in the human ID3 gene has been associated with atherosclerosis, however, its contribution to CBVD is unknown. This study uses a machine learning-based analysis of human brain microvessel transcriptome to identify novel ID3 gene regulatory networks in AD patients. Microarray data from human brain microvessels from 20 AD patients and controls were processed for normality. Probe set identifiers were mapped to Entrez identifiers, official gene symbols and gene names. Signal intensity values for each gene with multiple probes were averaged resulting in 21,000 unique gene annotations. Differential gene expression between case and controls was calculated by the Benjamin-Hochberg t-test ($p < 0.05$). ChIP-Seq analysis identified approximately 2,834 candidate genes bound by ID3 in endothelial cells. We discovered 38 ID3 bound target genes among 417 genes showing a 2-fold change between disease and non-disease brain microvessels. For Bayesian networks analysis, ID3 target genes were discretized in cases and controls. Variables such as age, gender, and disease were included into the Bayesian networks structure learning algorithm. Nine best structures were identified from running the learning algorithm nine times with different running times (three independent runs for 2 h, 4 h, and 8 h.). A Bayesian network structure identified at 8h was selected as the best scoring network comparing Bayesian Dirichlet scoring metric of all structures. The best scoring network showed a structural ID3 gene regulatory network interacting with age, gender, and disease. We discovered five key ID3 gene targets to be most influential to diseased brain microvessels (AMFR, BEX1, PARVG, PGM2L1, PRKACB). Furthermore, the best scoring network predicted a subject will have AD with a high probability (>0.999) when only three (BEX1, PGM2L1, PRKACB) of the five key genes are downregulated, and with a low probability (<0.001) when only PARVG was downregulated in human brain microvessels. Receiver Operating Curve analysis on ID3 gene network influence on disease resulted in an area under the curve of 0.75. Data driven machine learning is a powerful approach to predict ID3 causal gene networks involved in vascular disease. Given paucity of studies on human brain microvessels and small number of subjects in this study, a future goal will be to apply this method on a larger population with the hope of identifying blood-based biomarkers of vascular dementia.

1350 Assessment of Remediation of Arsenic from Water through Measuring Environmental Stressor Transcriptomic Gene Signatures

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Nuclear respiratory factor 1 (*nrf1*) is an environmental stressor gene, when exposed acutely by numerous environmental agents, such as temperature, physical activity, diet and accumulated metals it activates and regulates cellular functions. Molecular indices to measure Arsenic (As) in surface waters are currently unavailable. Here, we use the zebrafish which is a known sentinel for biomonitoring of aquatic environments. The objective in this study was to investigate *nrf1* transcription factor and its signature genes to identify orthologue signature gene networks which may be involved in the initiation and progression of cellular processes. To identify the common *nrf1* targets, we analyzed the ChIP-seq dataset from ENCODE. We then used bioinformatics and functional genomics to determine (As- *nrf1*) significant target genes comparing the treatment effects of low concentrations 15 (ppm) [As(V)] in liver hepatocytes while characterizing the cellular processes across 4 periods (8, 24, 48 and 96 h) of treatment time. The transcriptomic dataset of GSE3048 of 12 As treated and 12 control zebrafish samples was processed for normalization. A parametric *t* test, (*p*-value <0.05) between treated and control samples was used to determine differential expressed genes (DEGs) using the Benjamin-Hochberg False Discovery Rate (FDR). Dysregulated *nrf1* targets were determined based on a criterion of 2-fold change. During 8-96 h, we discovered 236 upregulated and 336 downregulated *nrf1* targets in As treated zebrafish. Furthermore, 8 h revealed 30 up and 72 down-regulated genes. A reduced number of downregulated genes were identified at 24 h, 60 up and 6 downregulated genes. Relatively equal number up and down regulated genes occurred at 48 h with 57 up and 62 downregulated. Broadly, we found genes at 96 h most dysregulated compared to previous times including 89 up and 196 genes downregulated. Using the functional gene ontology (GO) and (KEGG) pathways using DAVID pipeline, revealed that 72.6 % of the upregulated and 69.7% of the downregulated *nrf1* target genes matched DAVID enrichment process. The most significant upregulated GO terms across 8-96 h included metabolic processing p value (2.3E-4), cellular processing *p* value (9.5E-3) and developmental processing p value (9.5E-2). Using KEGG, *nrf1* targets play a key role in cell cycle, RNA transport, p53 signaling and glycerophospholipid biosynthesis. Our results show that the altered transcriptome responds to arsenic treatment in a sensitive manner. This provides a molecular approach to supplement traditional analytical measurements detailing a comprehensive pathway framework to monitor As in surface waters.

1351 Toward Predictive Toxicology—Computable Biological Network Models of Drug-Induced Liver Injury

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Drug interactions with cigarette smoke impact the absorption, distribution, metabolism, and excretion of and, consequently, the response to some drugs. The main mechanism behind drug interactions with smoke involves hepatic metabolism, where polycyclic aromatic hydrocarbons present in cigarette smoke induce cytochrome P450 isoenzymes. The induction of these enzymes results in accelerated metabolism of xenobiotics in smokers and a clinically significant decrease in the pharmacologic effect. Xenobiotic metabolism pathways are also involved in drug-induced liver injury (DILI), where high-energy conversion of compounds can lead to necrosis or apotosis in the liver. To investigate how smoking affects DILI, we created a causal biological network model that gathered available biological knowledge into a structured and computable format. The resulting model provides a connected view of the signaling crosstalk that is critical for not only prediction of drug

toxicity but also elucidation of pathophysiological mechanisms. The network model backbone consists of biological entities (nodes) and relationships between the nodes (edges). The model has an additional layer that consists of mRNAs known to be regulated by the entities in the model backbone. All model relationships are scripted in Biological Expression Language, which facilitates computation, and, instead of overlaying gene expression changes onto the model, the second layer is used to infer the activity of the backbone nodes from transcriptomic data. Each network edge was extracted from literature, annotated with the originating source (PMID) and biological context (i.e., species, tissue/cell type, and disease state). The main involved biological processes determining a cell death outcome include a combination of the following pathways: MEK/ERK, mTOR/p70-S6K, AKT and p38/MK2/HSP27. Combining our model with transcriptomic data from relevant animal and cell models exposed to a combination of smoke and DILI model compounds provides a means for mechanistic understanding and quantitative impact assessment of toxicant effects on the liver for smokers, non-smokers, and switchers to potential modified risk tobacco products. In addition, this validation process results in network models with predictive capacities that can be used in the context of predictive toxicology to investigate the impact of compounds on biological systems.

PS 1352 Utilizing Human Cell Lines for an Unbiased and Biologically Relevant Off-Target Screening to Support Different Therapeutic Modalities

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Unintended off-target activity remains a hurdle in drug discovery. The current approach of *in vitro* secondary pharmacology screening is often performed by pharmaceutical companies to assess unexpected binding and/or activity against a panel of off-target proteins. However, incomplete target coverage with only ~50 to 200 proteins in a typical panel, lacking biological context, and inadequacy to address specificity issues for emerging modalities such as RNA interference (RNAi) as well as Proteolysis Targeting Chimera (PROTAC), where off-target effects must be assayed changes in transcript or protein level rather than evaluation of binding or activity modulation, are some major limitations of this tool. To address this unmet need, we have developed a novel platform that allows unbiased protein profiling in biologically relevant settings. This platform can be used for off-target screening to support modalities beyond small molecules. Four cell lines were selected with a 73% coverage of the human genome based on transcriptome profiling results of CCLE (Cancer Cell Line Encyclopedia) cell lines. Global proteomics and detailed characterization were then carried out on these four cell lines. We were able to quantify a total of 10,627 protein with 10501 proteins from cellular fractions and 3146 proteins from secreted fractions. We further systematically investigated the protein targets that are implicated in cardiovascular, respiratory and central nervous systems with strong genetics and pharmacological evidence. Our proteomic platform was able to quantify 1828 proteins which are considered to be important to evaluate for drug safety assessment. The species conservation of these proteins between human, rat, dog, and monkey were also calculated, which could help provide preliminary clues on the translatability between species. This proteomic platform is best suited to screen off-targets for PROTAC and RNAi therapies, and with the aid of chemical biology, for small molecules as well. The platform may provide the opportunity to broadly assess functional essential proteins and to allow better forecasting the phenotypical consequence(s) that may lead to potential drug adverse events.

PS 1353 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) Effects of Hepatic One Carbon Metabolism during the Progression of Steatosis to Steatohepatitis with Fibrosis in Mice

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TCDD has been linked to the development of metabolic diseases including non-alcoholic fatty liver disease (NAFLD). One carbon metabolism (OCM) gene expression and metabolites levels, notably S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), are often altered in animal and human models of NAFLD. Changes in the SAM/SAH ratio affect methylation reactions including the biosynthesis of methylated metabolite and epigenetic marks. To investigate the dose-dependent effects of AhR activation on OCM, male mice were orally gavaged with sesame oil vehicle control or TCDD (0.01-30 µg/kg) every 4 days for 28 days. Histopathology showed increased hepatic fat accumulation, immune cell infiltration, bile duct proliferation, and collagen deposition. TCDD dose-dependently repressed adenosylhomocysteinase (*Ahcy*; ED_{50} 10.4 µg/kg), betaine-homocysteine S-methyltransferase (*Bhmt;* ED_{50} 11.2 µg/kg), cysthionine-β-synthase (*Cbs*, BMDL 0.1 µg/kg), glycine N-methyltransferase (*Gnmt*; ED_{50} 11.2 µg/kg), and methionine adenosyltransferase 1A (*Mat1a*; ED_{50} 4.5 µg/kg). Accordingly, protein levels of AHCY, BHMT, CBS, GNMT and MAT1A were decreased with increased levels of betaine, homocysteic acid, and methionine, while cystathionine, dimethylglycine, and the SAM/SAH ratio were reduced. Additionally, SAM-dependent biosynthesis pathways for polyamines and creatine were perturbed. Decreased expression of guanidinoacetate methyltransferase (*Gamt*, ED_{50} 14.6 µg/kg*)* increased guanidinoacetate in the liver and serum while decreasing urinary creatinine. Expression of ornithine decarboxylase (*Odc1;* ED_{50} 1 µg/kg) led to increased putrescine levels in spite of increased spermidine synthase (*Srm*; ED_{50} 0.35 µg/kg) expression. These results suggest AhR activation by TCDD leads to OCM disruption concurrent with the progression of steatosis to steatohepatitis with fibrosis. *This work was supported by the Superfund Research Program (NIEHS SRP P42ES04911) and the NIEHS Multidisciplinary Training in Environmental Toxicology Program (NIEHS EHS P2T32ES007255).*

PS 1354 High Content Screening of Toxic Response Phenotypes in Cells Derived from the Diversity Outbred Mouse Population

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Natural genetic variation significantly influences the physiological response to chemical exposure, and studying diverse populations can help decipher the underlying mechanism of response variability. With balanced allele frequencies, small linkage blocks, and ~90% of the genetic variation found in *Mus musculus*, the Diversity Outbred (DO) mouse population combines the experimental tractability of laboratory mice with high genetic mapping resolution. To establish a platform for cellular systems toxicology, we are generating a panel of primary fibroblast cultures from DO mice for *in vitro* High Content Screening (HCS) of arsenic exposure (sodium arsenite; $NaAsO_2$). The genotoxic effects of As exposure are a by-product of increased oxidative stress and inhibited DNA repair, which can be assessed at single cell resolution using the Operetta High Content Imaging system. Combined with a parallel *in vivo* sodium arsenite exposure, our cellular response phenotypes will ultimately be correlated to physiological responses and multi-omics analysis. Here we show our preliminary assessment of broad sense heritability for a range of cellular traits influencing the response to arsenic exposure using fibroblasts derived from the 8 isogenic founder strains of the DO population. Our results show that genetic background influences arsenic cytotoxicity, with the CAST/EiJ strain being particularly susceptible. Notably, the CAST/EiJ derived allele of arsenic methyltransferase (*As3mt*) has increased expression, identified through expression quantitative trait loci (eQTL) analysis, which may relate to the arsenic sensitivity. Taken together, these data show that our *in vitro* platform is sensitive enough to detect heritable differences cellular responses to toxic exposure, and mouse genetic reference populations such as the DO can be used for high resolution mapping and validation of the loci that influence toxic response.

PS 1355 Identification and Functional Characterization of a Novel Biomarker for Aryl Hydrocarbon Receptor (AhR) Activation

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The aryl hydrocarbon receptor (AhR) is required for normal vertebrate development and is also activated by several chemicals including polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-Tetrachlorodibenzodioxin (TCDD). AhR activation is well understood, but the roles of downstream molecular signaling events are largely unknown. Previously, we conducted transcriptomics in 48-hours post fertilization (hpf) zebrafish exposed to 16 PAHs. In addition to the expected cytochromes P450 like *cyp1a1* and *cyp1c1*, one of the most highly elevated transcripts by several PAHs was *wfikkn1* (WAP, kazal, immunoglobulin, kunitz and NTR domain-containing protein), a potential novel biomarker for AhR activation. The purpose of the present study is to investigate the functional role of *wfikkn1* in the AhR signaling pathway. Quantitative RT-PCR was used to demonstrate that *wfikkn1* is expressed in developing zebrafish from 2.5 to 120 hpf. Using *in situ* hybridization in 48-hpf zebrafish, *wfikkn1* mRNA expression was identified in several organs including the brain, otic vesicle, and the trunk. When AhR2 (ortholog of human AhR)-null and Wildtype zebrafish were exposed to DMSO or TCDD and the expression of *wfikkn1* was quantified at 48 and 120 hpf, the significant induction of *wfikkn1* was AhR2-dependent at both time points. To functionally characterize *wfikkn1*, a *wfikkn1* CRISPR-Cas9 mutant zebrafish line with a 16 bp exon deletion that produces a truncated protein was generated. The mutant zebrafish appear morphologically normal, but are hyperactive in a photomotor response assay at 120 hpf. To identify genes/pathways associated with *wfikkn1*, Wildtype and mutant

zebrafish were exposed to DMSO or TCDD, and mRNA from 48-hpf zebrafish was sequenced. Over 200 genes were differentially expressed ($p < 0.05$, $\log_2FC > 2$) between each pair of treatment combinations. The significant GO term processes associated with *wfikkn1* mutants included ATP-binding, immunoglobulin, and iron binding processes. ATP-binding, transmembrane, and protein kinase processes were uniquely altered in TCDD-exposed *wfikkn1* mutants compared to TCDD-exposed Wildtype zebrafish. We are currently conducting in-depth analyses of the RNA-sequencing data, and characterizing both *in vitro* Wfikkn1 protein activity and *in vivo* protein localization. Understanding downstream transcriptional events that occur upon AhR activation and play a role in toxicity pathways is necessary to accurately guide remediation strategies. *This study was supported by the SRP Grant P42 ES016465 and NIEHS Training Grant T32 ES007060.*

1356 A Multidonor Comparison of Human 3D Lung Epithelial Culture Models

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In vitro models of the human lung play an essential role in evaluating the toxicity of inhaled compounds and understanding the development of respiratory diseases. 3D organotypic models derived from lung epithelial basal cells and grown at the air-liquid interface (ALI) resemble the human airway epithelium in multiple aspects, including morphology, cell composition, transcriptional profile, and xenobiotic metabolism. Whether the different characteristics of basal cell donors, such as their ethnicity, age, sex, or smoking status, have an impact on the tissue characteristics and responses remains unknown. A major limitation of *in vitro* studies using cellular models from single donors is the potential bias towards a donor-specific response. In this study, we compared the characteristics of lung cultures grown at the ALI (bronchial and small airway) from different donors for morphology (histology and whole-insert immunostaining), functionality (transepithelial electrical resistance [TEER] and cilia beating frequency [CBF]), and metabolic capacity (cytochrome P450 [CYP] 1A1/1B1 activity). Bronchial and small airway cultures from 5 and 6 donors were evaluated from weeks 5 to 9 and weeks 8 to 11 after air-lift, respectively. The results showed differences in the thickness and cell-type composition of the epithelium across the donors and time after air-lift. TEER varied among the donors and, in general, progressively decreased over time. The frequency of ciliary beating increased in response to isoproterenol treatment irrespective of the donor or culture type. Untreated cultures presented low basal CYP1A1/1B1 activity, but treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for 48 h induced CYP1A1/1B1 activity regardless of the donor. In conclusion, our results show that lung epithelial cultures prepared from different donors present diverse morphology but similar functionality and metabolic activity. However, we detected certain variability in the degree of responses following stimulation, for example, with isoproterenol (for CBF) or TCDD (for CYP1A1/1B1 activity). Overall, our study shows that the characteristics of lung epithelial cultures grown at the ALI change over time, and the sensitivity of cultures from different donors to some stimuli is variable. Therefore, for experiments with primary cultures, it is pertinent to have appropriate controls for each assay and time-point measurement.

1357 Identifying Molecular Pathways Highly Sensitive to Chemical Exposure Using Bioinformatic Methods

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A major trend in regulatory toxicology of the 21st century focuses on molecular pathways in toxicity testing. How do we know what pathways to asses for their sensitivity to chemical exposures? Previous toxicological research has identified a number of such molecular mechanisms. We hypothesize, however, that there are other molecular mechanisms highly sensitive to a broad range of chemical exposures, which were not yet identified. To test our hypothesis, we extracted a gene list from the Comparative Toxigenomic Database data on chemical-gene interactions from only high-throughput experiments with human, mouse or rat cells/tissues/organisms. The list of chemicals was than manually annotated. Endobiotics, pharmaceuticals, drugs of abuse, chemical warfare and research chemicals were excluded from further analysis as they interact with important molecular pathways by design and the search of molecular mechanisms sensitive to chemical exposures may be clouded by inclusion of these compounds. Further analysis was conducted for a subset of chemicals used in agricultural, cosmetics, environment, as food components, industrial chemicals and pollutants. This dataset included 330,605 chemical-gene interactions from 1,294 publications and covered 554 chemicals and 26,200 genes. A number of activating and suppressing gene-chemical interactions were identified for every gene in this dataset, and the resulting lists of genes were used for functional enrichment analysis using Metascape and GSEA. The top five genes undergoing downregulation in response to a broad range of chemical exposures included 3 members of the growth-hormone (GH) - IGF signaling pathway (IGF1, GHR and IGFBP3). Four out of the top five genes were upregulated by many chemicals, including Phase I xenobiotic metabolizing enzymes (HMOX1, NQO1, CYP1A1, CYP1B1). Enrichment analysis demonstrates that pathways of response to toxic substance, cytokine mediated signaling, apoptotic signaling, oxidative stress response and unfolded protein response are activated by broad range of chemicals, while pathways involved in lipid metabolism were suppressed by many chemicals. Our unbiased approach has confirmed the importance of major toxicological pathways, but also identified other pathways not represented widely in toxicological research, including GH-IGF signaling, lipid metabolism and cytokine mediated signaling.

1358 Use of Transcriptomics to Identify Cosmetic Ingredients' Biological Activity: A Read-Across Exercise

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The objective of this work was to use transcriptional signatures to assess the biological activity of structurally related chemicals to define their biological similarity and with that, substantiate the validity of a read across approach usable in risk assessment. As a case study, we selected 4 short alkyl chain parabens: methyl-, ethyl-, butyl-, and propylparaben, as well as the main metabolite of these parabens, p-Hydroxybenzoic acid (pHBA). The comprehensive transcriptional response of MCF7 cells was evaluated (TempO-Seq, BioSpyder) after exposure to vehicle-control, each paraben or pHBA at 3 non-cytotoxic concentrations (1, 50 and 500 µM), for 6h. Differentially expressed genes (FDR ≥ 0.05, and fold change ±1.2≥) were identified for each of the parabens or pHBA at each dose of exposure and used to determine similarities between them. Each of the parabens is able to elicit changes in the expression of a large number of genes, as compared to controls, particularly at the highest dose tested. Importantly, the transcriptional profile elicited by each of the parabens shares a high degree of similarities across the category members. We identified 133 common genes whose expression is modified by each of the parabens in a significant manner in the same direction. pHBA elicited significant gene expression changes at the highest concentration evaluated (615 genes), however, these changes are mostly different than the ones elicited by any of the parabens. The highest number of genes commonly affected by the parabens was found between butylparaben and propylparaben, where 634 genes were in common. Pathway enrichment analysis (MSigDB v7.0) of the transcriptional profile for each paraben indicated a significant overlap in the up- and down-regulated pathways across the four parabens. This was indicative of their biological similarity, and thus the validity of the read across among the group. The highest similarity in biological activity was found between butylparaben and propylparaben. The top Hallmark pathways that are most up-regulated by these two parabens are: estrogen response early and late, and TNFA signaling via NFKB. While the top Hallmark pathways that are most down-regulated are: apical junction, NOTCH signaling, myogenesis, and hedgehog signaling. These pathways' similarities further support the conclusion that these two parabens are the most similar structural and biological analogs. *Supported by Cosmetics Europe.*

1359 From Input to Output: Understanding the Effects of *Epichloë coenophialum* Infection in Tall Fescue on the Fescue Plant and Grazing Beef Pathophysiology through Metabolomics and Microbiomics

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Epichloë coenophialum is a common endophyte in tall fescue and it increases plant vigor. However, *E. coenophialum*-infected tall fescue is referred to as toxic because the endophyte produces secondary metabolites (ergot alkaloids) that are detrimental to grazing livestock, resulting in fescue toxicosis (FT). We previously found toxic tall fescue (E+) grazing perturbs metabolic homeostasis and induces hindgut dysbiosis in beef cattle, but no study has assessed the impact of E+ on the tall fescue or rumen microbiota/metabolome, nor has this been integrated with fecal microbiota. We sampled novel, non-toxic (MaxQ) and E+ tall fescue plants, rumen contents, plasma, urine, and feces of Angus

steers grazing either MaxQ or E+ tall fescue for 28 days. 16S (bacteria) and ITS2 (fungi) rRNA sequencing and high-resolution metabolomics were used to assess changes in the microbiota and metabolomes resultant from *E. coenophialum* infection or exposure. E+ significantly affected the global microbiota profiles (bacteria/fungi) in all biological matrices. Plant fungi were the only core microbiota influenced by E+, with increased *Pucciniaceae*, *Pleosporaceae*, and *Dissoconiaceae*, and decreased *Mycosphaerellaceae*. Numerous plant bacterial OTUs were detected in the rumen and feces of both MaxQ and E+ steers, without much overlap for plant fungi. Most fungal taxa were significantly decreased by E+ across the GI tract. Bacterial *Lachnospiraceae*, *Coriobacteriaceae*, and *S24-7* families were increased in E+ rumen solids, liquids, and feces. The *Mogibacteriaceae* family was increased in both the rumen liquids and feces of E+ steers, while the *Veillonellaceae* was increased only in rumen liquids. Bacterial changes were more similar between the rumen liquids and feces than other biological matrices, indicating the overlap between the fore- and hindgut microbiota is driven by rumen liquids. Tropane, piperidine, and pyridine alkaloid biosynthesis was the top metabolic pathways significantly different between MaxQ and E+ tall fescue. Many metabolic pathways were affected by E+ in the rumen liquid (steroid and histidine metabolism), plasma (phenylalanine, tyrosine, and tryptophan metabolism), and urine (terpenoid backbone biosynthesis). Differential network analysis showed a highly correlated structure of OTUs and metabolites that are fescue cultivar specific, demonstrating global changes to the structure of the microbiota/metabolome in response to E+ grazing. Overall, this study provides a global view of plant and animal responses to *E. coenophialum* infection and E+ grazing, respectively, while deepening our understanding of FT etiology. *Support: USDA (NIFA 67030-25004).*

1360 Bioengineering of Artificial Thymoma Spheroids as a 3D Model for *In Vitro* Toxicity Testing Employing a Novel Microslot Nuclear Magnetic Resonance Technique

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The metabolic pathogenesis of thymic carcinomas (TCs) is poorly understood and adjuvant therapy has limited success in metastatic disease and tumor recurrence. Most studies on TCs use two-dimensional (2D) cell culture models, which are not considered as physiologically relevant, and consequently translation to the *in vivo* situation remains challenging. Tissue-specific architecture, based in part on interactions with the microenvironment is an essential component of tumors and may be better recapitulated in three-dimensional (3D) cell culture models. We established 3D models of two thymic carcinoma cell lines, TY82 and 1889c, in normal and serum free media using simple rotation for the former cell line, and 3D molds for the latter, which was co-cultured with Extracellular Matrix Protein (ECM). Characterization of the 3D models were performed using live imaging with a variety of dyes, as well as structural analyses. Subsequently, the effect of bortezomib, the most commonly used drug for thymomas, was compared between 2D and 3D models using traditional toxicity assays, as well as *in vivo* imaging. A technique was established using Nuclear Magnetic Resonance (NMR) that is suitable for measuring ultra-small volume samples, like 3D tumor models in order to perform metabolic profiling in these 3D models as a function of spatial position. For the first time, 3D spheroids could be made of reproducible size and quality using two thymic cancer cell lines. We could also produce spheroids using serum free media to decrease differentiation of the cells normally seen in this cancer type. Live/dead dyes showed that the cells in the untreated spheroids were viable; with evidence of a hypoxic core the larger the spheroid. Metabolic profiling of the 2D and 3D models for both cell lines were identified, Treatment with increasing concentrations of bortezomib showed higher IC50 values for the 3D models than 2D models with 2 different viability assays - CellTiter-Glo® and Prestoblue. Finally, we could establish the very first assay for noninvasive toxicity testing employing NMR. The present findings using 3D models of thymic cancer confirm that they are more resistant to drug-induced toxicity compared to 2D models. In addition, our novel approach using NMR allows for the measurement of small tissue-like models, which are normally not feasible with standard analytical techniques. The currently-available methods only provide a "snap-shot" of the measured time point and tend to be destructive, e.g. dissecting or optical cleaning of the specimen to gain 3D Information - a limitation we overcome with our current method using NMR spectroscopy.

1361 Comprehensive Histone, DNA Methylation, and mRNA Expression Analysis of Murine Liver Repeated Exposure to Chemicals—Percellome Project Update

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The Percellome Project aims at reinforcing and replacing the "safety factor" by comprehensively identifying the transcriptomic networks induced by xenobiotics. "Percellome" normalization method was developed to generate absolute copy numbers of mRNAs in a "per one cell" basis from the Affymetrix GeneChips. Data of mouse liver after a single oral gavage (4 time points (2, 4, 8, and 24 hours after dosing) x 4 dose levels (control, low, middle, and high), triplicate, 48 GeneChip data per chemical/organ from 48 mice) on 160 chemicals are compiled. In addition, data from newly designed repeated-dosing studies are added. This study uses 48 wild-type mice repeatedly given a same dose of a chemical for 4 or 14 days to create a "chemically induced transgenic state", and then, the next day, a single gavage of a chemical in four dose levels was given, and the liver was sampled at 2, 4, 8, and 24 hours thereafter. Up to now, GeneChip data on CCl_4, tributyltin, deet, clofibrate, valproic acid, acetaminophen, phenobarbital, thalidomide, 5-fluorouracil, acephate, imidacloprid, and diethylnitrosamine are obtained. Repeated dosing of CCl_4 suppressed the baseline expression of genes related to ER stress and also affected the rapid response to the final dosing. 14 day repeated dosing of valproic acid did not affected both the baseline expression and rapid response induced by the final dosing. As a result, we found that the effect of repeated dosing can be interpreted as a combination of two elements, i.e. baseline response (BR: gradual shift of the basal expression level) and transient response (TR: alteration of the magnitude and/or pattern of the quick response in 2 to 24 hours). To clarify the molecular basis of the BR and TR, whole genome bisulfite analysis (WGBA) and ChIP-seq against H3K4me3, H3K27me3, H3K27Ac, and H3K9me3 were performed on the liver samples of CCl_4 and Valproic acid repeated dosing studies. WGBA was validated by a F1 of C57BL/6 and Japanese domestic syngeneic JF1 mice that have 10 million SNPs so that maternal and paternal strands of imprinting genes can be clearly identified. In short, 14 days of CCl_4 treatment did not alter DNA methylation. On the other hand, ChIP-seq revealed that BR and TR of mRNA of some characteristic genes was in good correlation with histone modification. Detailed analysis of CCl_4 and Valproic acid studies will be presented.

1362 Early-Life TCDD Exposure Shapes Gene Expression and Chromatin Profiles across the Life Course of Mice

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2,3,7,8-Tetracholorodibenzodioxin (TCDD) is a potent environmental toxin that is generated as a byproduct of industrial operations involving high temperature processing of organic material. It enters into environmental systems as a constituent of solid waste and flue gas. In vertebrate systems, TCDD activates the AhR-mediated xenobiotic response which modulates transcription of numerous genes responsible for metabolizing toxic compounds. The World Health Organization recognizes links between early-life exposure to TCDD and late-onset pathologies including neurological disability, reproductive impairment, and increased cancer risk. Our goal is to understand the consequences of early-life TCDD exposure on the molecular state of multiple tissues. Mice were exposed to TCDD from preconception through gestation and lactation. Tissue samples were taken three weeks, five weeks, twenty weeks, and forty weeks after birth. From our measurements of transcriptional profiles, we show that gestational TCDD exposure shapes gene expression and open chromatin profiles both in the short-term and in the long-term. Substantial changes in molecular profiles accompanied early life exposure after accounting for signatures of tissue, age, and sex. Expression of 1497 genes and accessibility of 1571 loci were altered in liver combined over both sexes across all ages. The effects of TCDD differed dramatically between males and females. Though there were clear signatures of TCDD exposure at all age points, few changes observed at three weeks persisted into adulthood. Blood showed few differentially expressed genes at three weeks but substantially more in adult mice. However, there is no overlap between responses in liver and blood. We conclude that a complex cascade of gene regulatory events is set in motion by early-life TCDD exposure which results in long-term gene expression and chromatin accessibility differences in adult mice.

1363 Toxicokinetic Model Predicts Cellular Exposure in Organ-on-Chip Microdevices

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Organ-on-chip microdevices have proven useful to evaluate chemical toxicity in human cells cultured in 3D heterotypic microenvironments; however, such microdevices are often fabricated from polydimethylsiloxane (PDMS), which has high affinity for small hydrophobic molecules. When potential toxicants are tested in such devices, hydrophobic chemicals may partition into PDMS surfaces and reduce the dose that reaches the cultured cells, severely impacting dose-response curves. We investigated binding to PDMS surfaces for nineteen chemicals of interest in environmental toxicology – many of those have a range of uses from pesticides to pharmaceuticals and are linked to endocrine disruption or reproductive toxicity. For those chemicals that did bind PDMS, we fully characterized the chemicals' toxicokinetics to quantify chemical bioavailability in PDM-based microdevices to predict actual cellular exposures. Two representative test chemicals, propiconazole and molinate, which are known reproductive toxicants, were used to study the reproductive toxicity in fetal-membrane-on-chip microdevice. We experimentally observed that up to 90 ± 2% of propiconazole and 50 ± 10% of molinate bound to a PDMS test specimen with 1/e time-constants of 9.7 ± 1 and 13.6 ± 2 hr, respectively. The binding of molinate, but not propiconazole, was reversible, with 12 ± 2% able to leach back out in fresh aqueous solvent (time constant of 6.1 ± 1 hr). From these experiments, we extracted kinetic binding parameters to model the impact of PDMS-toxicant interaction on in-device cellular exposure under realistic flow conditions and device geometry. For an irreversible binder like propiconazole, a simulated acute dose of 10 μM for 4 hr yields an in-device cellular exposure of just ~1 μM, an order of magnitude less than the nominal dose. For a reversible binder like molinate, delivery of an acute dose of 2700 μM for 4 hr yields not only a reduced acute dose, but tails of extended low-dose exposure of up to 53 μM that lasted for tens of hours after the nominal dose. For such chemicals, where a significant proportion is lost into PDMS surfaces within a microdevice, this sequestering may yield false negatives in toxicant screens and could lead to incorrect conclusions from nominal-dose-response curves. Our toxicokinetic model demonstrates how to model such impact of PDMS-chemical interactions to predict chemical bioavailability and to estimate actual in-device cellular exposures in organ-on-chip microdevices.

1364 High-Content and -Throughput 3D Mouse Embryonic Stem Cell Spheroid Cultures for Characterizing the Developmental Toxicity Potency of Chemicals

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Prenatal developmental toxicity (PDT) testing is both animal- and resources-intensive. The mouse embryonic stem cell test (mEST) is widely used as one *in vitro* alternative to test for the potential developmental effects of chemicals and drug candidates. The traditional mEST model uses a "hanging drop" culture of embryonic stem (ES) cells that differentiate into beating cardiomyocytes in 3D embryoid bodies (EBs). This test evaluates chemical's potential to inhibit cell differentiation by manual quantification and microscopic analysis, a tedious procedure that is limiting broader application of this test due to low throughput and limited number of endpoints. The goal of the present study was to develop a higher throughput method based on high-content confocal imaging to characterize phenotypic changes of EB in response to test compounds. To achieve this goal, we focused on improving the workflow and throughput, as well as expanding the range of the phenotypes. First, we improved the workflow by generating functional EB in a bioprinting system by using magnetic nanoparticles and 384-well magnetic plate. Second, we evaluated additional phenotypes with a one-step staining procedure followed by rapid high-content confocal imaging and streamlined image processing algorithm that enables efficient comparison of different EB phenotypes. Finally, effects on the beating of functional EB are also recorded. The new high-throughput mEST method was tested with a number of compounds that have known embryonic toxicity. In addition, we screened a library of diverse environmental chemicals. Results showed that the performance of the optimized high-throughput mEST is comparable to the standard mEST and to *in vivo* embryotoxicity potency. In addition, different responses to tested compounds from 2D and 3D culture, as well as 3D cultures from different testing models (bioprinting plate and u-bottom low adhesion plate) were compared. In conclusion, the method developed in this study enables rapid multi-parameter evaluation of cytotoxicity in both 2D and 3D mEST systems and may be used as a screen for potential developmental toxicity of a wide range of compounds and mixtures. *This research was funded by P42 ES027704.*

1365 An *In Vitro* Physiologically Based Kinetic (PBK) Modeling-Based Testing Strategy to Predict Human Cardiotoxicity of Methadone

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Within the framework of developing novel alternative testing strategies to predict human toxicity, the aim of the present study was to investigate whether the cardiotoxicity in humans could be predicted by combining *in vitro* toxicity assays with PBK modelling-based reverse dosimetry. Methadone was used as a model compound because several human studies report cardiotoxic effects in clinical settings. The PBK model was developed based on metabolic parameters obtained from *in vitro* incubations with pooled human liver microsomes and parameters derived from *in silico* simulations and the literature. The *in vitro* methadone concentration-response curve based on prolongation of field potential duration was obtained by using a multi-electrode array (MEA) assay with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Using PBK modelling-based reverse dosimetry the *in vitro* concentration-response curve was converted to an *in vivo* dose-response curve representing effects of methadone on prolonged QT interval and related cardiotoxicity in humans, taking differences in protein binding in the *in vitro* and *in vivo* situation into account. Results show that cardiotoxicity predicted with the *in vitro* PBK approach was comparable to methadone toxicity data derived from published clinical studies with less than a 3-fold difference. Results also reveal that protein binding in plasma is an important factor in the sensitivity of different individuals for the adverse cardiac effects of methadone. Therefore the individual variation in plasma binding might provide an important factor in a personalized prediction of undesirable side effects of methadone treatment in the clinic. The results provide a proof of principle for an alternative testing strategy to predict *in vivo* electrophysiological cardiotoxicity for humans using the *in vitro* hiPSC-CM MEA assay integrated with PBK modelling-based reverse dosimetry. Given that cardiotoxicity is a leading cause of drug failure during drug development, the presented approach to predict *in vivo* cardiotoxicity in humans, might be a valuable addition to preclinical drug development testing strategies to detect cardiac safety liabilities.

1366 Assessment of Rat and Human Gut Microbial Metabolic Impact on the Isoflavone Daidzein and Its Metabolite *S*-equol Using Physiologically Based Kinetic Modeling

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The human body provides a habitat for vast microbial communities, the majority of which reside in the gastrointestinal tract, especially the distal gut. The gut microbiota plays a significant role in the health of the host due to a wide range of biochemical and metabolic activities that can affect the toxicity and bioavailability of xenobiotics. Current *in vitro-in silico* based testing strategies used for quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), however, do not include the gut microbiome. To assess the impact of gut microbial metabolism of daidzein for rat and human, an *in vitro* method was developed to quantify the kinetics of gut microbial metabolism in order to define a physiologically based kinetic (PBK) model that included the gut microbiota. The isoflavone daidzein was used as model compound, which is known to be converted by gut microbiota to dihydrodaidzein (DHD) as an intermediate metabolite and further to *O*-desmethylangolensin (*O*-DMA) and *S*-equol. Anaerobic *in vitro* incubations of rat and human* fecal samples were optimized to allow definition of the maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) of microbial metabolism of daidzein. To this end, fecal concentration and incubation time were selected from their respective linear ranges before testing a range of substrate concentrations. Apparent V_{max} and K_m for daidzein depletion and formation of the metabolites DHD, *O*-DMA and *S*-equol were quantified. Results showed that the gut metabolic catalytic efficiency for conversion of daidzein, expressed per kg bw, was more than 200-times higher in rat than human. Derived kinetics were used as input parameters for the PBK model, to predict both daidzein and *S*-equol plasma concentrations in rat and human. Predicted plasma concentrations of daidzein and *S*-equol were comparable to reported plasma concentrations in *in vivo* studies reported in literature. The described *in vitro-in silico* strategy allows prediction of *in vivo* consequences of intestinal microbial metabolism of xenobiotics, thereby contributing to 3Rs (Replacement, Reduction and Refinement) principles and 21st century toxicity testing strategies. **Approval has been obtained from The Medical Ethical Reviewing Committee of Wageningen University, the METC-WU.*

The METC-WU has reviewed the above-mentioned research file and believes this research does not fall within the remit of the Dutch 'Medical Research Involving Human Subjects Act'.

1367 Use of Human Polarized Intestinal Epithelial Monolayers for *In Vitro* Protein Hazard Characterization

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Previous work has demonstrated the utility of using human-derived intestinal epithelial cell (IEC) lines cultured as polarized monolayers on permeable Transwell® filters to differentiate between hazardous and non-hazardous proteins. The purpose of the current study was to explore exposure to various amounts of test proteins to determine effective concentrations that best resolve hazardous from non-hazardous proteins. The effects on IECs of several known hazardous (*C. perfringens* epsilon toxin, Listeriolysin O, *Phaseolus vulgaris* erythroagglutinin, *E. coli* Shiga toxin 1, *C. difficile* Toxin B and wheat germ agglutinin) and non-hazardous proteins (Ara-h2, β-lactoglobulin, fibronectin and RuBisco) were evaluated at different concentration ranges. Monolayer integrity effects (measured by TEER and flux of FITC-inulin or HRP) and cell viability (measured by MTT reduction and LDH release) were evaluated after 48-hour exposure of IECs to each dose of protein. Known hazardous proteins were largely identified as hazardous and could be effectively distinguished from non-hazardous proteins. Concentration range selection emerged as an important factor to consider when optimizing the ability to distinguish hazardous from non-hazardous proteins. This experimental platform may be expanded to serve as a component in the assessment of the safety of proteins expressed in agricultural biotechnology crops that are genetically modified (GM) to impart traits such as insect resistance or herbicide tolerance. Such proteins that have a history of safe consumption based on multiple *in vivo* high-dose acute oral toxicity, 28-day repeated dose, whole food/feed and animal performance studies, would be useful to evaluate in further assessing the utility of the cultured human IEC line monolayers as a component of the safety assessment for future proteins to be expressed in GM crops.

1368 Evaluating Mechanism of Aflatoxin B_1 and Fumonisin B_1 Interaction in *Caenorhabditis elegans*

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Contamination of mycotoxins is a world-wide food safety problem, and exposure or co-exposure to mycotoxins and their adverse effects has been a public health concern for decades. Two of the toxicologically significant mycotoxins, aflatoxin B_1 (AFB_1) and fumonisin B_1 (FB_1), were drawn specific attentions because their co-exposure induced considerably stronger toxic and carcinogenic effect than when exposed to either of the toxins alone in various animal models. However, the specific mechanisms pertaining to the co-exposure remain to be elucidated. Individually, AFB_1 is classified as Group 1 human carcinogen, and is found to be associated with increased risk to hepatocellular carcinoma and childhood stunting, while FB_1 is classified as Group 2B carcinogen, and is shown to be associated with esophageal cancer in various developing nations. In this study, the model organism, *Caenorhabditis elegans*, were treated with various concentrations of AFB_1, FB_1, and mixtures of both to evaluate various toxicological endpoints. Additionally, total RNA was extracted from worms treated with EC10 levels of the mycotoxins to assess alterations of lncRNA involved in toxic mechanisms. Using combination index method, we found that there is a greater-than-additive interaction between AFB_1 and FB_1 toxicity for all three tested toxicological endpoints, namely, growth, brood size, and lifespan. While FB_1 does not cause visible DNA damage, treatment with mixture of AFB_1 and FB_1 induces significantly greater amount of DNA lesion than treatment to the same concentration of AFB_1 alone. When comparing the lncRNA of AFB_1-FB_1 mixture to AFB_1-only exposures, the most differentially expressed lncRNAs are related to the genes *casy-1* (up-regulated) and *par-1* (down-regulated). The most differentially expressed antisense lncRNA are related to the mRNAs of *casy-1* (up-regulated) and *cpna-2* (up- and down-regulated). A few significant KEGG pathways affected by the additional FB_1 treatment include up regulation of cytochrome P450-related genes and down-regulation of alanine, aspartate, and glutamate metabolism. It may be plausible that FB_1 potentiates the toxicity of AFB_1 by potentiating the phase I metabolism involved in activating AFB_1 toxicity, while potentially down-regulating phase II and other detoxification pathways.

1369 Co-exposure of Bisphenol A and High Sucrose Diet Aggravates Diabetes-Mediated Renal Tubule Dysfunction Phenotypes in *Drosophila melanogaster*

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Sponsor: H. Gupta

The prevalence of diabetes has become a global epidemic that is magnifying the incidence of diabetic nephropathy cases. However, the traditional factors such as obesity, sedentary lifestyle, family history and excess carbohydrate are well recognized, but other risk factors such as environmental chemicals are poorly studied. Bisphenol A (BPA) is an environmental chemical that is used as a base compound to synthesize the epoxy resins and polycarbonate plastics. Humans are continuously exposed to BPA and in consequence, BPA has reported to be associated with the diabetes and reduced kidney function. However, limited experimental studies are reported on the role of BPA in diabetes mediated kidney dysfunction. Here, we have used Malpighian tubules (MTs) of *Drosophila* that share developmental and functional similarities with the mammalian renal tubule. The potential of BPA (0.1 and 1.0 mM) alone and co-exposure of BPA and high sucrose diet (HSD) (1.0 M sucrose) was investigated to induce diabetes and MTs dysfunction. Twenty days exposure of BPA (1.0 mM) alone to newly emerged flies resulted in imbalance of glucose homeostasis and perturbations in the insulin signaling, while co-exposure of BPA and HSD further elevated glucose level and perturbs insulin signaling in the exposed organism. Efflux activity and fluid secretion rate (functional assay) of MTs were found to be significantly declined in exposed organism at 1.0 mM BPA alone, while BPA + HSD treatment resulted in further decline in the above endpoints. Uric acid level was also found to be elevated in the BPA (1.0 mM) alone treatment of *Drosophila*. The effect was more drastic in BPA + HSD treatment condition. The study, therefore, suggests that co-exposure of BPA and high dietary intake of sucrose aggravates renal tubule dysfunction in exposed *Drosophila*.

1370 A Three-Dimensional Microfluidic Platform for Modeling Human Extravillous Trophoblast Invasion and Toxicological Testing

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Placental trophoblast cells invasion into the maternal uterus is an essential and complex event in the formation of the maternal-fetal interface. However, due to the complexity of the placental microenvironment, the development of the ideal *in vitro* trophoblast invasion model remains a challenge. We aimed to develop a microfluidic 3-dimensional (3D) model that recreates the invasion placental microenvironment. We used a 3D silicone polymer polydimethylsiloxane (PDMS) microfluidic chip, composed of a circular central compartment surrounded by two arched channels separated by a 50 µm barrier with 3 µm gap interspaced PDMS pillars. The 3D chip was coated with extracellular matrix (ECM), and seeded with human umbilical vein endothelial cells (HUVECs) and mCherry tagged first-trimester human placenta cells (HTR8/SVneo) in the central compartment and channels, respectively. We optimized the ECM pre-coating (gelatin *vs*. Matrigel *vs*. fibronectin), cell seeding density (10 - 30 million cells/ml) and culture medium flow speed (0.01, 0.05 and 0.1 µl/min). After optimization (fibronectin, 30 million cells/ml, 0.01 µl/min flow speed), we tested 1) the filtering ability of the endothelial-trophoblast barrier using a permeability assay and 2) HTR8/SVneo invasiveness using a chemoattractant (folic acid, FA) and invasion suppressor (tunicamycin, TUN). The permeability assay showed that the diffusion of FITC-Dextran is prevented between compartments when cells were at confluency. FA stimulated invasion of HTR8/SVneo cells into the fibronectin/endothelial layer, which was counteracted by TUN. Using the combination of this new 3D platform and fluorescent-tagged cells, we were able to harvest and sort by flow cytometry invading cells to further characterize specific gene expression patterns of invasion. This novel 3D microfluidic chip reproduces key elements of the human placenta barrier: a two-cell interface with a space barrier, a supporting ECM layer, and constant medium flow resembling *in vivo* shear stress. Using this platform, we were able to perform real-time monitoring, imaging, quantification, and harvest of invasive cells for detailed transcriptomic analyses. This new platform represents an advantage over 2D culture systems as it better resembles the placental microenvironment and may prove important for future toxicological screening testing in a complex organ such as the placenta. *Funded by SOT Alternative Research Grant and NIEHS R01ES027863 to A.V-L.*

1371 Cellular and Molecular Characterization of Brain Glucose Metabolic Changes in *Nauphoeta cinerea* Using Streptozotocin Treatment

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The brain depends on a regulated supply of glucose, and conditions that impair glucose availability to it severely impacts on its function. Insects, like vertebrates, depend on glucose as the main source of energy for the brain. However, little is known about how disruptions of glucose utilization by the brain and fat body modifies general metabolic activities in these important organs of cockroaches. We developed a novel model of streptozotocin-induced hyperglycemia in *Nauphoeta cinerea* and found raised glucose concentration and relative mRNA expression of glucose transporter 1, as well as, markers of oxidative stress in head tissues. Fat body glycogen and insect survival, as well as, acetylcholinesterase activity, triglyceride content and viable cells in head homogenate were reduced. The decrease in glycogen, triglycerides and MTT reduction may indicate a disruption in glucose utilization by the head and fat body of insects after toxic treatment with streptozotocin. The insects were able to increase their glutathione s-transferase activity and total thiol levels, possibly via activation of nuclear factor erythroid 2 related factor, to attain a balance between the generation of reactive oxygen species and antioxidants. Other researchers have shown that the haploinsufficiency of glucose transporter 1 is linked with neurodegenerative conditions like Alzheimer's disease, our data present *Nauphoeta cinerea* as a viable model for studying the cellular responses to brain and fat body glucose alterations, and we phylogenetically demonstrated conservation between *N. cinerea* glucose transporter 1 and those of other Insects.

1372 Hepatocyte Spheroid Models and High-Throughput Transcriptomics as New Approach Methodologies in Predictive Toxicology Screening

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Three-dimensional (3D) spheroid models with improved tissue-like functionality may offer better *in vitro* safety assessment of chemicals. Combining these models with emerging low-cost, high-throughput transcriptomics would enable systematic characterization of chemical induced effects on biological systems. We have developed spheroid liver models of human progenitor cell line, HepaRG, and primary rat hepatocytes in 384-well formats. HepaRG and rat spheroids exhibit several hallmarks of polarized hepatocytes and tissue-like functionality. Activities of xenobiotic metabolism enzymes, CYP1A2, CYP2B6 and CYP3A4 were 2 to 20-fold higher in HepaRG spheroids compared to the sandwich-cultured primary human hepatocytes. Similarly, specific activities of CYP1A2 and CYP3A enzymes in rat hepatocyte spheroids remained at the same levels as freshly isolated hepatocytes even after 14-days in culture, displaying longevity in drug metabolism activity. High-throughput gene expression analysis using the S1500+ gene set was performed on HepaRG spheroids treated with ten concentrations of a reference set chemicals for 96-hours in repeated exposure regimens. Baseline gene expression and biological pathway responses showed significantly higher enrichment scores in spheroids compared to monolayer cultures. Exposure to aflatoxin B1 and benzo(a)pyrene showed activation of genes and pathways related to their metabolism and anticipated downstream signaling events such as cell cycle, p53 signaling, DNA damage and cancer. BMDExpress was used to calculate point of departure for individual genes and pathways associated with concentration-related molecular perturbations. Activation of nuclear receptor pathways (CAR/RXR and PXR/RXR) was evident with lower concentration of phenobarbital and rifampicin preceding onset of stress-related pathways at higher concentrations. Similarly, biologically relevant pathways associated with exposure of chlorpromazine and valproic acid were activated at sub-lethal concentrations. Differences in gene and pathway-level expression between liver injury chemicals (trovafloxacin, troglitazone and tolcapone) and their structurally similar analogs was observed. The initiation of molecular events associated with chemical exposure and progression of events that lead to adverse outcome effects were clearly evident with high-throughput transcriptomics visualized on ten-point concentration-response analysis. *In vitro* models utilizing spheroids and data-rich approaches may offer better resolution in identifying molecular level perturbations of biological systems and prediction of hazardous substances

1373 Scaffold-Free 3D *In Vitro* Model of Ovarian Toxicity Exhibits Physiologically Relevant Hormone Response

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Ovarian granulosa cells are the functional unit of the ovary with steroidogenic activities and play a pivotal role in regulating reproductive health of females. Humans are consistently exposed to thousands of environmental chemicals that have been detected in female follicular fluid. Many of these chemicals have never been tested and can have direct disruption on granulosa cell function, thus result in toxic effects in reproductive health. Therefore, there is an urgent need to develop effective platforms that can efficiently screen a vast number of chemicals for their potential health impact. The present study aim to develop a high throughout platform to screen for chemicals that may impact the steroidogenic activities of human granulosa cells. Three-dimensional human granulosa spheroids were generated by seeding human ovarian granulosa-like tumor KGN cell line into the Microtissue Inc agarose platform. KGN cells self-assembled into scaffold-free spheroids inside the agarose molds within 24 hours of cell seeding, and expressed the steroidogenic CYP19 aromatase enzyme. We demonstrated that in the presence of testosterone, KGN spheroids have significantly greater estradiol and progesterone production than KGN cells cultured in two-dimension (2D), with an estradiol production rate that is comparable to primary human granulosa cells culture in 2D as previously reported. Hormone production by KGN spheroids also had a dose-response increase in the presence of follicle stimulating hormone (FSH) and dibutyryl-cAMP, both of which stimulates the steroidogenic function of CYP19. Overall, these functional KGN spheroids are highly promising for predictive *in vitro* toxicity testing, and we envision using them to screen chemicals in follicular fluid including polychlorinated biphenyls and per- and polyfluoroalkyl substances.

1374 A Systematic Variation of Experimental Conditions to Help Harmonize Zebrafish Early Life Stage Bioactivity Screening

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Zebrafish are used extensively to discover novel pharmaceuticals, investigate mechanisms of chemical action and for human disease model research. Although the zebrafish model is gaining acceptance for 21st century toxicology research, there is a lack of harmonized experimental approaches for its use in developmental toxicity screening, limiting full model adoption. A few identified sources of variability include: the presence/absence of the chorion (an acellular membrane that could pose a barrier to chemicals), the photoperiod, and the precise dosing regimen (daily renewal v. static exposure). To address these variables, a systematic approach was taken to alter each experimental condition for nine chemicals and a single nanomaterial. These materials were intentionally selected to represent a range of physiochemical properties including photo-lability and log K_{OW}. Utilizing the Tanguay lab early life stage exposure paradigm, the ten compounds were assessed for bioactivity across three exposure conditions: dechorionated v. chorion on, dark exposure v. light/dark cycle exposure, and static exposure v. daily solution renewals. Embryos were exposed at 6-8 hours post-fertilization (hpf) and screened for 22 morphological and two behavioral endpoints at 120 hpf. The bioactivity profiles for each chemical were compared across all conditions by calculating concentrations which produced a 50% response (EC_{50}). Results show chorion status affects the bioactivity of four of the compounds tested and exposure regimen affects bioactivity for six of the compounds tested. The results of this study will provide information necessary to help investigators tailor zebrafish assay conditions to better match the physiochemical properties of test agents. This should increase data reproducibility, confidence, and acceptance, moving the field one step closer to utilizing the powerful zebrafish screening platform to broadly predict chemical bioactivity.

1375 High-Throughput Assessment of Compound-Induced Proarrhythmic Effects in Human iPSC-Derived Cardiomyocytes

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Drug-induced QT interval prolongation and Torsades de Pointes (TdP) arrhythmias are the leading causes for drug withdrawals from market and compound attrition during drug development. To assess clinical potential

drug-induced TdP, the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) is a new cardiac safety testing paradigm that includes *in vitro* assays using human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM). In order to adopt this paradigm, we developed a high-throughput hiPSC-CM assay using calcium-sensitive dyes that exhibit fast kinetic fluorescence detected by high-speed fluorescence imaging platform. We evaluated concentration-dependent responses of 28 pharmacological agents linked to low, intermediate, and high TdP risk categories. Compound-induced proarrhythmic effects such as changes in beating rate and durations of these 28 agents were quantified and reported by monitoring changes in intracellular Ca2+ oscillations. Characterization of calcium oscillations allows us to evaluate complex waveforms and events. In addition to beat rates and duration, we observed compound-induced proarrhythmic effects or peak prolongations at concentrations correlated to clinically-relevant concentrations of QT prolongation and TdP arrhythmias. We also characterized waveform irregularities and more than 20 other dose-dependent read-outs. Moreover, we assessed cellular and mitochondrial toxicity of these 28 compounds in the follow-up assay using high-content imaging to simultaneously monitor acute & chronic dosing effects of structural cardiotoxicity with multiple toxicity indicators. Taken together, we demonstrate that our high-throughput assay can provide an *in vitro* drug-induced proarrhythmia assessment as a part of the cardiac safety paradigm encouraged by CiPA initiative and strengthens pharmacological safety profiles of drug candidates.

PS 1376 The Cross-Laboratory Testing and Evaluation of a Cardiac Microphysiological System

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Cardiotoxicity, and a poor understanding of human pharmacokinetics have long been cited as major reasons for safety-based pharmaceutical withdrawals. This is in part due to the limitations of existing animal and *in vitro* models to adequately represent human biology. To address these limitations, microphysiological system (MPS) models of cardiac tissue are being actively developed; however, the adoption of these and other MPS has been slow due to lack of wide-spread use of these devices outside of the developer laboratories. Here, we conducted a cross-laboratory evaluation of a 3D, human, ventricular MPS which had previously demonstrated cell alignment, realistic mass transport properties, and IC_{50} and EC_{50} values more consistent with human responses than traditional cellular-scale studies. First, the cardiac model was transferred from the University of California Berkeley to Texas A&M University and tested to ensure cell alignment, as well as determine baseline beating parameters (beat rate, time interval, and contraction and relaxation velocities). Next, a dose escalation treatment with cisapride, verapamil, and isoproterenol (1nM - 10uM, 15-minute exposures) was applied to monitor changes these cell responses. Finally, the cardiac model was challenged with sorafenib, carbamazepine, or atenolol over a period of 1 week at a constant exposure (0.01-100uM) to determine sub-acute effects on the tissues within the chips. These studies were carried out in parallel with standard 2D well-based cultures as a comparator to determine the differences in sensitivity for a variety of beating parameters. Additionally, multiple cardiomyocyte cell sources (in-house differentiation vs. commercial) were evaluated in the cardiac MPS, as lot-to-lot variability is often a major challenge for any *in vitro* model. Here, we highlight differences in culturing methods (2D vs. 3D), cell sourcing, and data collection methods (phase contrast image analysis vs calcium-flux) in deriving human relevant point of departures. We also show that effects on beating parameters can be observed and quantified, and IC50 values determined. Results indicate that cells cultured in 3D exhibit higher sensitivity to drug-induced effects, at times deviating from baseline values at concentrations 10-1000X lower than 2D counterparts. Additionally, parameters like beat velocity (contraction and relaxation) are more difficult to quantify in an adherent 2D monolayer due to cell adhesion on the surface of the plate, but could serve as a useful metric in 3D culture. This model could potentially be used as a tool to estimate efficacy and dosing of drug candidates prior to clinical studies. *This research was funded by grants from NIH (U24 TR001950 and U24 TR002633).*

PS 1377 Evaluation of AAV Transduction Efficiency among Novel Human and Monkey *In Vitro* Liver Models

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The emerging field of Gene Therapy (GTx) relies heavily on the use of adeno-associated viruses (AAV) as a delivery vehicle for transgenes. Recent non-clinical toxicology studies with high-doses of AAV have demonstrated acute, severe hepatoxicity and systemic inflammation in non-human primates (NHP). To better understand the mechanisms and potential risk for AAV-induced liver toxicity in human clinical trials, we evaluated novel *in vitro* culture systems which more closely resemble *in vivo* liver. Sandwich culture hepatocytes (SCH) are widely used in the pharmaceutical industry to study drug metabolism but suffer from poor AAV transduction efficiency, mostly attributed to lack of liver architecture and cell type heterogeneity. In this work, we compared AAV transduction of SCH with three dimensional (3D) spheroid liver microtissues (LiMT) and microfluidic-enabled liver-chips comprised of either human or cynomolgus monkey-derived cells. SCH were seeded on collagen-coated dishes and overlaid with Matrigel according to standard practices. The LiMT were prepared with primary human or monkey hepatocytes and human non-parenchymal cells (InSphero). Liver chips were seeded with primary human or monkey hepatocytes and liver sinusoidal endothelial cells (Emulate Bio). Cell viability was assessed across all models by LDH or ATP assays and demonstrated AAV to be essentially non-cytotoxic at viral concentrations up to $1x10^6$ MOI gc/cell and $3x10^{10}$ gc/mL under static and flow conditions, respectively. AAV transduction efficiency was assessed by quantifying eGFP transgene expression through high content imaging, ddPCR, immunohistochemistry and confocal microscopy. Infection of LiMT with AAV at time of aggregation, as opposed to post-aggregation, led to higher transduction and deeper penetration of virus into the spheroid. Preliminary analysis suggests that liver chips exhibited higher eGFP mRNA expression compared with SCH. Full transcriptome RNA-seq was conducted to compare each *in vitro* model to normal liver. These evaluations are expected to identify an AAV-transducible and physiologically relevant *in vitro* model that will enable further mechanistic research to understand the relevance of high-dose AAV toxicity in NHP as it pertains to human safety risk in GTx programs utilizing AAV.

PS 1378 Drug-Induced Seizure Liability Mechanism of Action Analysis by Sequential Principal Component Analysis and Clustering Using Multiple Metric Parameters from Microelectrode Arrays Data of Primary Rodent Neurons

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Detection of drug-induced seizure liability is challenging. Dissociated cultured neurons and micro-electrode array (MEA) system is a potential non-clinical *in vitro* seizure liability screening model. The effects of known compounds with defined seizure liability profiles on neuronal activity on the MEA, with the goal to identify activity signatures that associate with specific liability profiles that could be used to screen unknown compounds for seizure liability. Primary cortical neurons were prepared from fetal Wistar rats, 18 days post-coitum, and then seeded on 48-well Classic MEA plates for extracellular recording using Maestro-1. Sixteen seizure positive compounds (4-aminopyrdine, amoxapine, amoxicillin, bupropion, chlorpromazine, diphenhydramine, enoxacin, linopyrdine, pentylenetetrazole, phenytoin, picrotoxin, pilocarpine, strychnine, theophylline, tiagabine and tramadol) and seizure negative acetaminophen were added to the cells (n = 6) at 5 concentrations for each compound to see phenotypic changes on spontaneous electrical activity in neural networks, after 19 days of culture. Mechanism of action (MOA) analysis was carried out by principal component analysis (PCA) and clustering sequence using 73 multiple metric parameters, which are calculated by AxIS Metric Plotting Tool. The clustering well classified inhibitory neuron suppression type compounds (penthylenetetrazole, picrotoxin and strychinine) and the other seizure positive- and negative compounds. However, enoxacin alone was classified in the seizure negative cluster, combination treatment of nonsteroidal anti-inflammatory drugs, and NSAIDs (felbinac, flubiprofen or ketprofen) induced seizurogenic phenotype of enoxacin. The clustering result showed combination of enoxacin and NSAIDs were located in the inhibitory neuron suppression compound's cluster as reported seizurogenic MOA of new quinolone antibiotics and NSAID combination previously. We have a plan to do structure-seizure liability relationship MEA analysis of new quinolones in the next step. MEA technology has the potential to predict the seizure liability of drugs and drug candidates with MOA by PCA and clustering using multiple metric parameters.

1379 A Novel Co-culture Model of Prostate Epithelial and Stromal Cells

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The 3-dimensional (3D) *in vitro* models have the potential to improve the physiological relevance, and the development of a normal prostate model in 3D can greatly advance the risk assessment of endocrine disruptors. The goal of this project is to develop an *in vitro* prostate model for high-throughput exposure-led safety assessment to enable robust safety decision making for endocrine disruptors. The development and normal function of the prostate is highly dependent on the interaction between stromal and epithelial cells; therefore, in this study, we established a co-culture model composed of the red fluorescent protein (RFP)-labeled benign human prostate epithelial (BHPrE) cells and green fluorescent protein (GFP)-labeled benign human prostate stromal (BHPrS) cells grown in scaffold-free hydrogels. The optimal 3D co-culture condition was defined for cell seeding density, epithelial to stromal ratio and cell culture medium composition. In the current model, epithelial differentiation was observed in both fluorescent images and hematoxylin and eosin (H&E) stained microtissue sections. Cytokeratin 5/6 (CK5/6) and Cytokeratin 18 (CK18) immunohistochemical staining further revealed the heterogeneity of the epithelial population. Image analysis pipelines for morphological feature identification were developed in the software of Harmony and CellProfiler. 3D image analysis-based cell counting and cellular volume analysis suggested no significant epithelial or stromal cell proliferation or cell death within 7 days. Among the hundreds of morphological features output from the pipeline analyses, we have identified a cluster of morphological features of the epithelial compartment, including sphericity, ellipticity, maximum thickness, and maximum cross-section area to combine into a composite description of response to androgen and anti-androgen treatment for screening. In summary, this BHPrE and BHPrS co-culture prostate model shows reproducible morphological features of differentiated prostate epithelium, which is responsive to androgen and anti-androgen treatment, allowing this co-culture prostate model to serve as an endocrine disruption chemical screening platform.

1380 Developmental Toxicity Induced by Heavy Fuel Oil Extracts Is Mediated by Their 3- to 7-Ring PAHs with a Role for the Aryl Hydrocarbon Receptor (AhR) Activation

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Heavy fuel oils (HFO) are heavy petroleum substances (PS) that may contain high amounts of 3- to 7-ring polycyclic aromatic hydrocarbons (PAHs) and are known to be able to induce developmental toxicity in animal experiments. In contrast, highly refined base oil (HRBO) consisting primarily of saturated hydrocarbons tested negative for developmental toxicity. In an attempt to develop and apply alternative testing strategies for assessing the developmental toxicity of highly complex PS, the present study uses the ES-D3 cell differentiation assay of the embryonic stem cell test (EST) to assess the embryotoxicity potency of DMSO-extracts of 8 HFO, possessing different CAS number and a systematic variation in their 3- to 7-ring PAH content, and 1 HRBO, containing no aromatics. In the EST, all DMSO-extracts of HFO induced concentration-dependent inhibition of ES-D3 cell differentiation and this potency was appeared to be proportional to the level of 3-7 ring PAHs they contain. Moreover, all HFO extracts also showed aryl hydrocarbon receptor (AhR)-mediated activities, as tested in the AhR CALUX assay. On the other hand, the HRBO extract tested negative in both assays. To investigate whether activation of the aryl hydrocarbon receptor (AhR) plays a role in the HFO-induced inhibition of ES-D3 cell differentiation, ES-D3 cells were co-incubated with HFO extracts and the AhR antagonist trimethoxyflavone (TMF). Results showed that the HFO-induced inhibition of ES-D3 cell differentiation was counteracted by the addition of TMF, confirming the role of the AhR in mediating the observed effects in the EST. Furthermore, a good correlation (R^2:0.99) between the *in vitro* and the *in vivo* developmental toxicity potencies of the HFO extracts understudy was obtained. In conclusion, our findings corroborate the hypothesis that developmental toxicity induced by some PS, e.g. HFO, is mediated by their 3-7 ring PAHs content and that the observed *in vitro* developmental toxicity is partially AhR mediated.

1381 Zebrafish Early Life Stages as a Preclinical Model to Predict Acute Oral Toxicity

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Zebrafish (*Danio rerio*) early-life stages have emerged as a promising model in preclinical drug screening so as to bridge the current gap between *in vitro* and rodent studies. The purpose of this work was to evaluate the acute toxicity of substances with different GHS categories using zebrafish early-life stage to determine LC_{50} (lethal endpoints) and EC_{50} (sublethal endpoints) values and compared with *in vivo* acute oral toxicity data from literature. Thirteen substances (2-propanol, Acetaminophen, Acetylsalicylic acid, Atropine sulfate, Cadmium II chloride, Dichlorvos, Ethanol, Glycerol, Mercury II chloride, Propranolol, Sodium chloride, Sodium oxalate, Verapamil HCl) were evaluated by Fish Embryo Acute Toxicity (FET) test based on OECD guideline no 236. Groups of 20 embryos were exposed in triplicate to a negative control and five concentrations of different chemicals. The test was performed in climate chambers at 26 ± 1.0°C with a 12:12 h light:dark cycle. Lethal and sublethal effects were evaluated during 96 h of exposure to determine LC_{50} and EC_{50} values. A linear regression-model using the log-transformed of these values was generated for the prediction of LD_{50} from LC_{50} values. This model resulted in the following equation Log (LD_{50}) = 0.4659 x log (LC_{50}) + 1.736 with R^2 of 0.7597. The correlation between CL_{50} and DL_{50} values was confirmed testing LQFM021, a promising anti-inflammatory, analgesic and antinociceptive agent. Considering acute oral systemic toxicity assessment in Swiss female mice was found lethal dose between 300 < LD_{50} < 2000 mg/kg, which classified as Category 4. The DL_{50} predicted with FET test was of 647.58 mg/kg, which also classified as Category 4. LQFM021 showed significant sublethal effects such as delayed of yolk sac ($CE_{50\text{-}96h}$ 77.68 mg/L), equilibrium ($CE_{50\text{-}96h}$ 77.27 mg/L) and edema cardiac ($CE_{50\text{-}96h}$ 71.28 mg/L). These results can be compared to acetylsalicylic acid, atropine sulfate, propranolol and sodium oxalate (category 4), which present the same effects like delayed of yolk sac ($CE_{50\text{-}96h}$ 21.28 - 1445 mg/L) and equilibrium ($CE_{50\text{-}96h}$ 8.8138 - 885.5 mg/L). Our results suggested that zebrafish early-life stages can be used as a preclinical model to predict acute oral toxicity.

1382 Yeast *Saccharomyces cerevisiae* as a Model Organism to Study Environmental Chemical Exposure and AhR Signaling

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The aryl hydrocarbon receptor (AhR) is a soluble ligand-activated transcription factor and a member of the Per-Arnt-Sim (PAS) superfamily. As cellular regulator, the AhR plays an important role in the metabolic and physiological adaptation to the external environment. To study the effects of environmental chemical exposure, we developed an AhR biosensor using the yeast, *Saccharomyces cerevisiae* as a model system. A series of optimization studies consisted of: yeast growth phase characterization, defining time of exposure, determining media composition and multi-well plate configurations. Conditions were established that allow reproducible analysis of AhR ligand activity in high throughput. While initially being less sensitive than CALUX. The sensitivity of the yeast system was enhanced by the introduction of the proteins ARA9 and ARA3 to the system. The yeast system is rapid, low cost, and displays pharmacological similarity to mammalian systems.

1383 A Human iPSC-Based *In Vitro* Neuronal Network Formation Assay for Regulatory Developmental Neurotoxicity Testing

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The spatiotemporal orchestration of key neurodevelopmental processes (KNDP) is essential for brain development. If at least one KNDP is impaired due to exposure towards a compound during a critical period of neurodevelopment, an adverse outcome is expected. To study developmental neurotoxicity (DNT) hazards, animal-free new approach methods (NAM) have been developed, which model certain KNDP *in vitro* and *in silico*. One of these KNDP is the formation and function of neuronal networks (NNF), the ultimate, functional readout for nervous system function. For studying NNF, the NNF assay based on hiPSC-derived human glutamatergic excitatory and GABAergic inhibitory neurons, as well as primary human astroglia (NeuCyte, USA) was developed.

These pre-differentiated cells were seeded in standardized ratio on 48-well microelectrode array (MEA) plates. We let the cells differentiate for one week until we received first recording as an internal control for a 'positive' (firing) well. After one week of differentiation in absence of test compounds, cell and network activity was measured via the assessment of spike-related (e.g. mean firing rate), burst-related (e.g bursting ratio) and network-related parameters (e.g. network synchronicity) using the Axion Maestro Pro System. These measures served as the baseline for subsequent neurodevelopmental testing. Treatments started on active wells by exposing from day 7 to day 35. With this protocol, the positive compounds BIS-I and Mevastatin were established as endpoint-specific controls for the assay. Both compounds reduced the values of the above described parameters in a time- and concentration-dependent manner. Data from control wells demonstrate low plate-to-plate and well-to-well variability of the assay. Currently, a set of 35 pesticides, that are known to either affect or not affect brain development based on the rodent DNT guideline study (OECD TG426), is tested in the NNF assay. These results are presented. The human NNF assay will be a valuable addition to the current DNT *in vitro* testing battery as neuronal network formation converges on multiple neurodevelopmental KE like neurite outgrowth, dendritic spine formation and synaptogenesis.

1384 Automated Image Analysis of Cell Morphology for High-Throughput Toxicology Assays Using Deep Learning Methods

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High throughput toxicology screening assays often employ morphological analysis of pictomicrographs acquired from 96- and 384 multi-well cell culture plates. Thousands of images generated from these assays require time-consuming and tedious manual analysis. Moreover, human interpretation of cell culture morphologies remains semi-subjective with reader-to-reader variation that can be a barrier for regulatory adoption of morphological observations. To automate this process, we have developed deep learning methods to automatically classify cell morphology images acquired with phase-contrast microscopy. We have trained a convolutional neural network (CNN) to perform binary and multi-binning classification. The binary classifier assigned assay images into healthy (comparable to vehicle controls) and altered (not comparable to vehicle-controls) classes with >98% accuracy; the multi-binning classifier assigned assay images into healthy, stressed and altered classes with >93% accuracy that accounts for cell loss. Our dataset comprised 2,156 and 2,254 high-resolution (1392x1036 pixels) images from proliferating and differentiated cultures of HepaRG cells. The dataset was manually annotated into four classes, i.e. Cell Loss, Stress, Abnormal and Normal by expert annotators. In our previous work, CNN showed better accuracy than traditional machine learning methods. In this study, we have focused on testing and fine-tuning various CNN architectures, including ResNet 34, 50 and 101. In addition, we used the watershed algorithm and a combination of image processing approaches to count the number of healthy and dead cells, which indeed correlated with the 4 image classes. To visualize regions in the images classified by CNN model, we employed Class Activation Maps (CAM). This allowed us to better understand the inner workings of the neural network and led to further optimizations of the algorithm. Finally, we are exploring various methods to perform dose-response analysis of the classification scores that may eventually be used to compute benchmark dose concentration upon chemical exposure. Together, these results demonstrate that automated deep-learning based image classification of cell morphology pictomicrographs can yield reproducible and highly accurate classification of changes associated with cellular stress upon chemical exposure.

1385 Systematic Application of New Approach Methods to Uncover the Mode-of-Action of Herbicides and Fungicides with Unanticipated Toxicological Properties: A Joint EU-ToxRisk and Syngenta Case Study

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Fast and reliable identification of toxic effects is crucial in the development of new active substances. New approach methods (NAMs) enable high throughput screening without the costs and ethical concerns of animal tests. Also, NAMs provide insights into underlying mechanisms of toxicity, which can be useful in screening new active substances. Syngenta provided 10 compounds that are either commercially available or research compounds that were discontinued due to developmental and reproductive toxicity (DART) effects. A selection of the EU-ToxRisk *in vitro* test battery was applied to determine if they can be used in hazard assessment or screening of new active substances for weed and disease control in cereals. 20 CALUX assays and 14 HepG2 BAC-GFP reporter gene assays were chosen to study activation of (nuclear) receptors and cellular signaling pathways. Cell type specific assays included high content imaging in primary human hepatocytes (PHH) and cell migration (UKN2) or neurite outgrowth assays (UKN4) in human dopaminergic neuronal cell lines. Hierarchical clustering was applied to the points of departure (PoDs) that were determined in each test system. This analysis revealed three distinct clusters of activity patterns that correspond to the known modes of action of the 10 case study compounds (1) Brequinar; (2) ACCase inhibitors; (3) fungicides of unknown mode of action. The results also gave insights into potential mechanisms underlying the toxicities observed *in vivo*. Group 1 showed a high activity pattern across the *in vitro* tests with activation of endocrine receptors, steroidogenesis and oxidative stress pathways and inhibition of neuronal function. Group 2 displayed a low activity pattern with activation of cellular stress response pathways and some effects on neuronal cells. Group 3 showed a moderate activity pattern across test systems with activation of several cellular stress response pathways and effects on neuronal function and viability. Combined but not individual analysis of the results gave the best match of clusters to known activity mode. Mechanisms of toxicity will also be analysed through transcriptomic changes in liver (PHH) and kidney (RPTEC/TERT1) cells. Computer models will be applied to determine the oral equivalent doses of the PODs for extrapolation to the human situation in hazard assessment.

1386 High Content Imaging Analysis for the Testicular Toxicity Prediction Using an 87-Compound Library in a Three-Dimensional Testicular Cell Co-culture Model

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Novel *in vitro* alternative testing approaches for reproductive is essential, supporting the principle of reducing, replacing and refining, as well as reducing the time and costs associated with costly reproductive toxicity testing. In our previous study, we developed an *in vitro* three-dimensional (3D) testicular cell co-culture model. We observed a strong correlation between the *in vitro* IC50 and *in vivo* rLOAEL with 32 tested compounds and confirmed that this model was able to discriminate reproductive toxicants. In this study, we integrated this testicular cell co-culture model with single-cell phenotypic analysis using high-content imaging to further validate this *in vitro* model using an 87-compound library. This 87-compound library provided by the National Toxicology Program (NTP) included known and suspected toxicants such as flame retardants, drugs, industrial chemicals, polycyclic aromatic hydrocarbons (PAHs), pesticides as well as negative controls. The co-culture cells were treated with 87 compounds in a wide range of doses (1-100 µM), and the multi-parametric high-content analysis (HCA) was conducted. High-content assays included multiple endpoints of adverse outcome pathways including nuclear morphology, DNA synthesis, DNA damage, and cytoskeleton structure. The data from the single-cell-based image analysis were geometrically averaged for each well for statistical analysis. Compounds were ranked by potency on individual endpoints, such as half-maximal effective concentration

(EC50) and selectivity of adverse outcome signaling pathways for hazard prioritization. Dose-dependent changes of these known reproductive toxicants were observed. Furthermore, the compounds displayed differential toxicity profile and demonstrated their unique mechanisms of toxicity. None of the presumptive negative controls and vehicle controls were shown toxicity in this culture model. This study not only validates the efficiency of this *in vitro* model but also tests the robustness and relevance of the model system with multiple endpoints that associated with adverse outcome pathways of the male reproductive system. *Supported by R44 ES027374 funding.*

1387 Methylmercury Myotoxicity during Development

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Methylmercury (MeHg) is a ubiquitous environmental contaminant and potent developmental toxicant. Prior studies showing outcomes of cognitive and motor deficits stemming from prenatal and early life MeHg exposures has solidified the notion that MeHg has a preference for neural targets. Nonetheless, recent work from our lab now establishes that processes of muscle development are sensitive to MeHg. Using the *Drosophila* model, we have shown that developmental exposure to MeHg compromises subsequent formation of adult indirect flight muscles (IFM) during pupal metamorphosis, inducing a "balling" or myosphere phenotype consistent with a failure in muscle-tendon attachment in late-stage pupae. This finding is consistent with a prior genome wide association study (GWAs) in *Drosophila* for susceptibility to MeHg that resolved candidate genes that are involved in muscle structure and development. Two such factors were *kon-tiki (kon)* and *inflated(if)*. *Kon* encodes a Chondroitin-sulfate-proteoglycan-4 (CSPG4) homolog, while *if* encodes an alpha-integrin, both being conserved cell adhesion proteins essential in a number of morphogenic processes in vertebrates. Both *kon* and *if* are expressed in IFM muscle and are required to form attachments across the myotendinous junction (MTJ). We hypothesize that MeHg targets these factors, resulting in aberrant MTJ formation and compromised muscle function. Exposure to 10 µM MeHg is seen to elevate *kon* transcript expression during a specific window of pupal development that precedes timepoints when the overt myosphere phenotype occurs. In contrast, no change in *if* transcript level is seen across pupal stages with MeHg. Transgenic overexpression of *kon* phenocopies the myosphere phenotype induced by MeHg. Knocking-down *kon* expression with RNAi restricted to an early pupal developmental window is able to partially rescue myosphere formation induced by 10 µM MeHg, consistent with the notion that failure in the MTJ is mediated through MeHg influence on *kon*. Additional studies in progress aim to discern the particular sequence of event(s) of MTJ formation that fail as a consequence of MeHg. Resolving these underlying mechanisms of failure in muscle attachment are likely to shed light on conserved mechanisms of muscle morphogenesis that are targeted by MeHg in vertebrates.

1388 Defining the Reproducibility and Applicability Domain of devTOX *quick*Predict, a Human Pluripotent Stem Cell-Based Developmental Toxicity Assay

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To establish confidence in new approach methods (NAMs) and enable their use in a regulatory setting, it is necessary to assess a NAMs accuracy and reproducibility, as well as define its applicability domain, in terms of both chemical and biological space. Over 100 chemicals have been evaluated with the devTOX *quickPredict* (devTOX^{qP}) assay, which predicts the developmental toxicity potential of a chemical based on changes in human iPS cell metabolism. The assay predicted the developmental toxicity potential across this diverse set of chemicals with 87% accuracy (88% sensitivity, 86% specificity). Within individual chemical classes (i.e., pharmaceuticals or pesticides), assay accuracy ranged from 81% to 94%, demonstrating the broad applicability of the assay. The reproducibility of the predictive model was evaluated using independent replicates of three chemical treatments (carbamazepine, n=34; methotrexate, n=34; thalidomide, n=9) conducted by multiple technicians with multiple iPS cell lines, freeze lots and reagents over the course of 5 years. The interpolated developmental toxicity potential (dTP) values (determined using the devTOX^{qP} predictive model) were within two standard deviations of the mean for each of the chemicals, demonstrating that the assay endpoints are reproducible over time. To understand the applicability domain of the assay, the results were separated into different pharmacological categories and performance was assessed. The assay's sensitivity in the different pharmacological categories ranged from 50% to 100% and provides insight into the assay's biological applicability domain. For example, developmental toxicants classified as channel, kinase, and transcription modulators and DNA modifiers were predicted as developmentally toxic with 100% sensitivity. In contrast, receptor modulators were predicted with 50% sensitivity, and were highly dependent upon whether the iPS cells expressed the specific receptor being modulated. These data demonstrate the importance of understanding a NAM's biological system, its strengths and its limitations. Taken together, these data demonstrate the accuracy, reproducibility and broad applicability domain of the devTOX^{qP} assay and support its use as an alternative to animal models for developmental toxicity testing.

1389 Implementation of a Human-Based Medium-Throughput Screening Assay in a Developmental Neurotoxicity (DNT) Testing Battery

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It is widely accepted that *in vitro* methods allow faster and cheaper toxicity testing for hazard identification than traditional animal experiments. However, except for some areas (e.g. skin sensitization, genotoxicity) those methods are not accepted by regulatory agencies. In the field of developmental neurotoxicity (DNT) there is consensus that a large number of chemicals needs to be tested to fill the current knowledge gap. Such a plethora of compounds cannot be tested according to current animal guideline studies. It was therefore recognized by stakeholders from academia, industry and regulators, that there is an urgent need for a regulatory accepted testing strategy that allows DNT evaluation of large numbers of chemicals in a reasonable time. Therefore, under the guidance of the European Food Safety Authority, we set up DNT test methods (NPC1-5) as part of a larger, international testing battery, that cover a variety of neurodevelopmental key events (KE) over developmental time. NPC test methods are based on aggregated primary human neural progenitor cells (hNPC) called neurospheres, which allow chemical screening on hNPC proliferation (NPC1), migration (NPC2), neuronal (NPC3), and glial (NPC5) differentiation. These assays are set up in a medium-throughput format in an automated experimental workflow with fully automated image analyses and evaluation. We are currently evaluating the effects of >100 compounds in a concentration-dependent manner on hNPC development using this workflow. Here we present the screening results of NPC1-5 and demonstrate how this model, that already produced reliable toxicity data with much smaller number of compounds and manual handling in the past, performs with a much larger set of substances in an automated experimental workflow. We show test methods performances, concentration-response curves for selected compounds, prediction models and benchmark concentrations of the chemical test set. The outcome of this screening project will further contribute to an OECD guidance on the use and interpretation of this DNT test battery. Here, an emphasis will be put on the relation between *in vitro* effective concentrations and human *in vivo* exposure levels for risk assessment and thus will accelerate the development and use of DNT *in vitro* test methods for regulatory purposes.

1390 Human Liver-on-a-Chip as a Model for Biotransformation Studies

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Organ-on-chip concepts are promising to improve current *in vitro* models that often fail to mimic organ complexity, and as such may facilitate the replacement of animal models. The aim of this study was to develop a liver-on-a-chip model based on human liver HepaRG cells with a high relevance for biotransformation studies. To test the performance of the system, we extensively characterised the chip model versus the conventional "static" HepaRG model and against primary hepatocytes grown under static conditions. After seeding, the HepaRG cells were allowed to proliferate for two weeks, followed by two weeks of differentiation. During differentiation and another six weeks thereafter, the cells were characterised. Cellular morphology was analysed weekly using confocal microscopy, showing the presence of hepatocytes, biliary cells, and bile canaliculi during the entire period similar to the static HepaRG model. Metabolic activity of CYP1A1/2 and CYP3A4, with and without stimulation with TCDD (induction of CYP1A1) and rifampicin (induction of CYP3A4), was evaluated weekly using an EROD and Luciferin-IPA assay, respectively. Gene expression of various metabolic enzymes and transporters was assessed at the start and end of the full differentiation period in the HepaRG cells and in

the primary hepatocytes. The chip and static system showed clear differences in basal metabolic activity with a higher CYP1A1/2 activity and lower CYP3A4 activity in the chip system. After stimulation, the activity of both CYP1A1/2 and CYP3A4 was lower in the chip system, but CYP1A1/2 activity in the chip resembled that of primary hepatocytes. For both HepaRG systems, CYP3A4 activity was lower than in primary hepatocytes. In summary, the HepaRG cells can be successfully grown under dynamic conditions in a chip maintaining their morphology. Metabolic activity was shown to be influenced by the dynamic conditions in the chip compared to the static system, but the observed differences were not unidirectional. Compared with primary hepatocytes, the dynamic system showed a similar CYP1A1/2 activity, but lower CYP3A4 activity after enzyme activity induction. Results show that the HepaRG dynamic chip system maintains metabolic activity up to six weeks after full differentiation and is thus a suitable system for biotransformation studies.

1391 Transcriptomic Data of Liver and Kidney Cells Treated with Valproic Acid and Analogues to Support Read-Across Assessment

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A read-across assessment usually starts with a list of initial source compounds sharing structural and physico-chemical properties. The finally selected source compounds also should exhibit similar toxicodynamic and kinetic properties. The conclusion on similar dynamic properties is often challenging, in particular because mechanistic data are usually not available. Legacy animal repeat dose oral exposure data show longer-chain analogues, like Valproic acid induce microvesicular liver steatosis as primary toxic effect with renal weight loss at higher dosing. Short-chain analogues, like 2-Ethylbutyric acid and Pivalic did not show adverse liver or kidney effects. We treated the human liver cancer cell line HepG2 and the human renal proximal tubule cell with 18 carboxylic acids (six concentrations) and conducted transcriptomic analysis using TempO-Seq. Unsupervised clustering of differentially expressed genes (DEGs) revealed clustering of steatotic versus non-steatotic carboxylic acids using the HepG2 model. Activity increased with increasing side chain length. A similar response was found with RPTEC/TERT1 cells. Further comparative analyses like pathway analyses among all analogues in both HepG2 and RPTEC/TERT1 cells are discussed. In summary, by studying the mode of action on a transcriptional level, we anticipate to support risk assessment by providing qualitative similarity data on a mechanistic level. By testing different cellular models, we will also learn more about the minimal scope needed to conclude on shared modes of action. *This work received funding of the EU-ToxRisk project (Grant agreement No 681002).*

1392 A Weight-of-Evidence Approach for Androgen Receptor Conservation across Vertebrate Species

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The US EPA's endocrine disruptor screening program (EDSP) is tasked with assessing thousands of chemicals for their potential to adversely impact human health and the environment through perturbation of endocrine pathways. Traditionally, chemical screening is performed using a tiered toxicity testing strategy that includes whole-animal studies. This approach, however, has proven challenging due to the extensive time, resources, and animals needed to evaluate a single chemical for endocrine activity. Therefore, the EDSP has been transitioning towards the use of *in vitro* high-throughput screening (HTS) techniques to rapidly and efficiently prioritize chemicals for further testing. Despite their utility, the ability of these mammalian-based HTS assays to accurately reflect chemical interactions with non-mammalian targets remains uncertain. One goal of the EDSP is to evaluate biological pathway conservation across taxa to understand how broadly these HTS results can be extrapolated to non-mammalian species. Identification of chemicals that modulate the androgen receptor (AR) is of interest to the EDSP because many chemicals have androgenic activity that can disrupt the endocrine system by mimicking or antagonizing natural hormones. Therefore, the objective of this study is to gather weight of evidence for AR pathway conservation across species using a combination of *in silico* structural comparisons and systematic review of available toxicity literature, both *in vitro* and *in vivo*, to determine whether current AR HTS assays are predictive of activity in other vertebrates. Results of this work provide lines of evidence toward structural conservation of AR across vertebrate species and suggest that chemicals shown to interact with AR ligand binding domain in mammalian HTS assays should behave similarly in non-mammalian vertebrates.

1393 Historical Comparison of Vehicles for 3T3 Neutral Red Uptake Phototoxicity Test (3T3NRUPT)

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The *in vitro* 3T3 NRU PT is used to identify potentially phototoxic test substances that are induced after exposure to light. The test evaluates phototoxicity by the relative reduction in viability of cells exposed to the chemical in the presence versus the absence of light. Substances identified by this test are likely to be phototoxic *in vivo* after systemic application and distribution to the skin, or after topical application. Depending on chemical solubility, different solvents may be used as vehicles to dilute the substance for the test. Test substances are diluted from a top concentration of 1 mg/ml or the limit of solubility in a decimal geometric series for a total of eight concentrations in an aqueous vehicle, and applied to a 3T3 mouse fibroblast cell line monolayer plated in two 96-well tissue culture plates. One plate is exposed to 5J/cm^2 of solar simulated light (+SSL), while the other plate is placed in the dark (No SSL) (50 min.). The effective concentrations at which the viability of the cells is reduced to 50% of untreated cells (EC_{50}) is calculated for each condition, and the photo-irritation factor (PIF) is defined as a ratio of the EC_{50} of the +SSL cells to the No SSL cells. These values are used to determine potential phototoxicity, where a PIF of less than 2 is considered to be non-phototoxic, a PIF greater than 2 and less than 5 indicates a probable phototoxin, and a PIF greater than 5 is phototoxic. A positive control, usually chlorpromazine (CPZ), must have a PIF value of greater than 6. We present 14 years of historical data +SSL and No SSL, and PIF values of the positive control, chlorpromazine (CPZ), in the recommended vehicles of Hanks' Balanced Salt Solution (HBSS), 1% dimethyl sulfoxide (DMSO) in HBSS and 1% ethanol (EtOH) in HBSS to compare the effects of vehicle choice on CPZ on the 3T3 NRU PT. The average PIF values of chlorpromazine in the three vehicles are similar: 44.5 (HBSS), 34.6 (1%DMSO), and 37.3 (1%EtOH). Although the dilution vehicle in which the substances are tested has some potential to affect these values, it is concluded that all three vehicles are acceptable without compromising study results.

1394 Development of an *In Vitro* Assay for Inhalation Toxicity

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A shift in toxicity testing from *in vivo* animal models to *in vitro* human cell-based models is a major focus of current research, due to the high cost, low throughput, ethical concerns, and questionable human relevance of *in vivo* testing. *In vitro* testing for point-of-contact inhalation toxicity is a particular challenge, and in doing so two factors should be considered. The first is the physiological relevance of the cell culture model used in the test. A more complex organotypic culture, such as the EpiAirway (MatTek) primary-derived, differentiated bronchial epithelium, may better replicate adverse outcome pathways, whereas a simpler model system, such as the BEAS-2B immortalized bronchial epithelial cell line, may be more applicable to high-throughput testing. The second consideration is the route of exposure. Whereas exposure of submerged cultures to compounds in solution is a commonplace approach, compatible with many *in vitro* assays and amenable to scaling for high-throughput testing, exposure of cultures grown at the air liquid interface to compounds in gas or vapor form may better simulate *in vivo* inhalation exposures. To better understand how these parameters affect testing results, we have developed a four-quadrant matrix of testing approaches that combines simple (BEAS-2B) or complex (EpiAirway) cell culture models with in-solution or vapor-phase exposures. As an initial test compound, we have chosen methyl iodide for its well-understood and direct mechanism of action in airway epithelia (depletion of glutathione and subsequent cytotoxicity). We found little difference between the cell culture models in sensitivity to methyl iodide-induced cytotoxicity. This was true when treated in solution (IC50s: 2.6 mM for BEAS-2B and 3.1 mM for EpiAirway) or as vapors (IC50s: 972 ppm for BEAS-2B and 1191 ppm for EpiAirway). However, we found that the in-solution sensitivities could not predict the vapor-phase sensitivities when converted to equilibrium vapor concentrations. The vapor IC50s calculated from solution IC50s were approximately three times higher than the measured vapor IC50s. This may be explained by the fast evaporation of methyl iodide from exposure medium, with a half-time of ten minutes. Therefore, in-solution exposure was of limited duration, whereas vapor-phase exposure could be maintained for longer durations. We conclude that vapor-phase exposure better mimics in-life exposure scenarios for volatile compounds.

1395 An *In Vitro* Alveolar Epithelial Cell Model Recapitulates *In Vivo* LRRK2 Inhibitor-Induced Increases in Lamellar Body Size

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Alveolar type II cells (ATII) contain lamellar bodies (LB) which synthesize and store lung surfactants. In animals, pharmacological inhibition or knockout of leucine-rich repeat kinase 2 (LRRK2) leads to increases in both the size and number of LBs in ATII cells. The consequences of these changes to the health or function of the lung are unknown. To understand the impact of pharmacological LRRK2 inhibition in lung, we developed an *in vitro* assay to examine changes in LBs in ATII cells. In this model, primary human alveolar epithelial cells (AEC) were cultured on Matrigel and collagen-I coated transwell inserts resulting in the display of similar phenotypes to ATII cells *in vivo*. By day 3 in culture, microvilli and LBs were apparent, and by day 7, after transition to air-liquid interface (ALI), LBs fused with the cell membrane and surfactant was released into the apical chamber. After 7 days under ALI conditions, typical ATII mRNA markers including ABCA1 and the surfactant proteins B, C and D were strongly upregulated, compared with cells cultured on standard plastic culture conditions (2D). The alveolar type I (ATI) cell marker AQP5 was also upregulated, suggesting a heterogeneous population of alveolar epithelial cell types. We also noted increases in both mRNA and protein levels of LRRK2 from cells cultured in this model, relative to 2D; a finding which agrees with reports of LRRK2 expression in lung being the highest within the human body. Additionally, we demonstrate that treatment of AECs with a potent LRRK2 inhibitor reduces endogenous phospho-LRRK2 and significantly increases LB size; an effect which is also seen in non-human primate lungs treated with the same compound. In summary, this model could expand understanding of LRRK2 and surfactant biology with the potential to provide clinical translation of the biological effects observed with LRRK2 perturbation in animals.

1396 Approaches in the Evaluation of Equipment Used for *In Vitro* Phototoxicity Testing to Ensure Proper Assay Performance and Regulatory Compliance

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Generation of accurate and compliant data hinges on monitoring assay performance. Specific assay equipment are required for photosafety assessments as outlined in OECD TG 432: *in vitro* 3T3 NRU Phototoxicity test and in the ICH S10 Document. We demonstrate the importance of evaluating the solar simulator and UVA meter achieve proper assay performance. A comparison of the UV emission spectra of two solar simulator bulbs showed variation in the UVA/UVB range with a noted abnormal shift in the 300-360 nm range for one bulb. To evaluate if the differences could affect assay performance, three reference compounds were tested. The absorbance peaks for hexachlorophene (non-phototoxic), chlorpromazine (phototoxic), and amiodarone (phototoxic) are predominately >200 nm to < 350 nm. In the bulb with an abnormal spectrum, amiodarone did not produce a significant shift in toxicity to fall into a probable phototoxic or phototoxic prediction as outlined in OECD TG 432. Our initial findings justify the importance of a spectral analysis measurement prior to use in an assay. UVA broadband meters are used to measure light output to ensure proper irradiance of cells equivalent to approximately 5 J/cm^2. Five different radiometers were sent for calibration - the historical meter and four others for comparison. During assay use, the historical meter read 1.7 ± mW/cm^2, but none of the new meters had a similar reading. One new meter was selected due to its stability and reproducibility; and after multiple comparative readings were taken, a conversion factor range of 1.25-1.27 was assigned. To confirm appropriate light exposure and assess tolerance of the cell line to light exposure, a cell sensitivity check was performed. The Balb/c 3T3 cells were exposed up to 9 J/cm^2 without adverse effect and the viability was >80% relative to control group. In addition, a positive control, chlorpromazine, was conducted to assure appropriate irradiance to elicit a phototoxic response. The results were compared to a historical database with a current Mean Photo Effect (MPE) range of 0.394 – 0.749. In summary, these approaches are important in providing confidence in the test system to produce reliable, accurate, predictive, and quality data.

1397 Generation of Machine-Learning Models to Anticipate Endocrine Disruption

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Endocrine disruption is a major focus of toxicology research, and thus human estrogen and androgen receptors are key targets of interest. Downstream effects of receptor activation are difficult to anticipate without expensive, time-consuming *in vitro* and *in vivo* testing, so the Environmental Protection Agency (EPA) has prioritized alternative methods. Recently, the EPA has used high-throughput ToxCast/Tox21 screening data across relevant targets and processes to construct mathematical models capable of predicting the likelihood of estrogen or androgen pathway agonism/antagonism of a chemical. One limitation of these studies is the requirement of *in vitro* data for prediction; in contrast, machine learning methods are capable of prospective prediction from molecular structure alone. Particularly, Bayesian machine learning models (BMLMs) have shown broad applicability to drug discovery and toxicology applications. The current study describes the generation and evaluation of several groups of BMLMs using androgen and estrogen receptor data, including *in vitro* ToxCast/Tox21 data, EPA mathematical model output scores, and binary data considering bioactivity and cytotoxicity. Group performance was evaluated by cross-referencing external predictions of *in vitro* and *in vivo* reference chemicals. These predictions were evaluated to produce an overall active/inactive classification for each chemical, and classifications were then compared to the results reported by mathematical model studies published by the EPA. BMLM prediction accuracies ranged from 86-93% for reference chemicals of androgen and estrogen receptors, for both agonist and antagonist action. Exploration of other machine learning algorithms, including deep learning, was conducted on training datasets for further comparison. This study demonstrates that prospective prediction using ToxCast/Tox21 assays is achievable at the same level of accuracy seen in recent EPA publications, on the basis of molecular structure alone.

1398 Quantification of the Total, Free, and Intracellular Concentration of Selected Compounds *In Vitro*

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Research in *in vitro* dosimetry is needed to improve *in vitro - in vivo* extrapolation in order to translate *in vitro* derived effect-concentrations to *in vivo* doses. Commonly used nominal concentration (C_{Nom}) may not represent the effect concentration initiating toxic events. Here, Balb/c 3T3 cells were incubated with test compounds to determine the medium (C_{Med}) and cellular concentrations (C_{Cell}) over time. A steady-state mass balance model (Armitage et al., 2014, Fischer et al., 2017, Kramer et al., 2012) was implemented to estimate predicted concentrations (C_{Pred}) which were compared to experimental results. Balb/c 3T3 cells were treated for up to48 h with Acetaminophen (APAP), Ketoconazole (KET), Methyltestosterone (MT) at subtoxic nominal concentrations of 60, 15 and 14 µM. Corresponding analytical concentrations were at 64.6, 12.6 and 12.7 µM, respectively. Total C_{Med} was corrected by the protein binding, determined by rapid equilibrium dialysis (RED). HPLC-MS/MS analysis was used for quantification of the compounds in medium, cells and RED samples. After 48 h, C_{Cell} for APAP, KET and MT were at 34.7, 21,0 and 260.5 µM and C_{Med} were 62.6, 8.8 and 6.3 µM. Comparison of C_{Med} and C_{Cell} to C_{Pred} for APAP, a fold difference of 0.1 and 4 is observed. For MT, fold differences of 0.1 and 47 for C_{Med} and C_{Cell} were observed. C_{Med} and C_{Cell} detected for KET show fold differences of 0.4 and 0.5 and are therewith close to C_{Pred}. Free C_{Pred} were found in close agreement to the experimental data. In conclusion, the work provides an insight into the quantification of *in vitro* dosimetry concepts in a diffusion-based *in vitro* assay versus a model-based approach.

1399 Integration of Transcriptome Data into the Hazard Assessment of Volatile Compounds: A Read-Across Approach

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Read-across is one of the most often used alternative methods in chemical risk assessment. It is often a challenge to conclude on shared toxicodynamic properties within the grouped substances based on observed adverse effects from *in vivo* studies. New models providing mechanistic information might be useful to support this assessment. ExITox II (Explain Inhalation Toxicity) addresses this question with compounds to which humans are exposed via the inhalation route. Human lung alveolar epithelial (A549) cells were exposed to five different read-across groups of airborne compounds, including gases, liquid aerosols and nanoparticles. The read-across groups comprise structurally related compounds. After repeated inhalation exposure aliphatic diamines, aldehydes and alkyl acrylates induced mainly pulmonary inflammation in rodents, whereas vinylester showed in addition hyperplasia and the nanoparticles led to fibrosis. Cytotoxicity was measured using mainly air-liquid exposure with the P.R.I.T.® ExpoCube® setup and repeated exposure. Whole transcriptome analysis (TempO-Seq) revealed DEGs (differentially expressed genes) per read-across groups in a dose dependent manner, with some nanoparticles having only very few DEGs. With the geneXplain platform, enriched TFBS (transcription factor binding sites) and the corresponding transcription factors (TFs) were identify. These TFs were the input for a master regulator (MR) analysis, the postulated MRs may function as marker/key molecules for the investigated substance groups. Finally group specific functionalities of DEGS, TFs and signaling pathways were described. The experimental validation of the proposed MRs is ongoing by quantitative RT-PCR. Unsupervised clustering was used to group the compounds according to their DEG/MR pattern. Further comparative analysis among the read-across groups providing more evidence on the association of DEGs/MR and signaling pathways with the observed adverse *in vivo* outcome will be discussed. We intend to support hazard assessment within a read-across approach by providing more evidence on shared toxicodynamic properties using transcriptome data. *This work has been funded under grant agreement FKZ 031 L0120A (BMBF program).*

1400 Development of Heterogeneous 3D Tumor Spheroids Models for *In Vitro* Drug Testing

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The *in vitro* 3D model system provides an alternative to the inaccurate 2D system and animal models that are expensive and time-consuming. The 3D model system encompasses the complexity of ECM and the physiological relevance of an *in vivo* system. We designed and discovered a 19-residue self-assembling hydrogel-forming peptide in which this peptide directly assembled into a hydrogel by mixing the peptides with Minimum Essential Medium used in cell culture, and this hydrogel has desirable properties for 3D cell growth with a final peptide concentration as low as 1 mM (0.17%). This 3D model provides easy and safe cell isolation for further cell physiological and pathophysiological studies. The goal of this project is to synthesize the hydrogel peptide derivatives and support the 3D multicellular growth for the human tumor cell lines. Epithelial, endothelial, and fibroblast cells were co-cultured along with the hydrogel peptide derivatives in a suitable culture media. The results showed that the heterogeneous 3D tumor spheroids were generated successfully for three cancer types - breast, colorectal, and prostate. Analysis of heterogeneous 3D spheroids of colorectal cancer have a 12-fold increase of beta-catenin, compared to controls without hydrogel peptide derivatives in which these derivatives are linkers of epithelial and endothelial cells. Heterogeneous spheroids of prostate epithelial and endothelial cells have a 53% increase in spheroid size compared to the control without hydrogel peptide derivatives. Heterogeneous 3D spheroids for breast epithelial and endothelial cells have a significant increase in growth compared to controls. Overall, heterogeneous 3D spheroids with hydrogel peptide derivatives - linking epithelial, endothelial, and fibroblast cells - modulate the growth rate of the spheroids, creating a more accurate model for drug testing.

1401 Engineered *In Vitro* Technologies to Identify Risk Factors in Breast Cancer

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Microenvironmental stress created by reactive oxygen species (ROS), a consequence of exposure to environmental factors and individual genetic make-up have been known to play a role in breast cancer risk and when not cleared by antioxidant mechanisms lead to oxidative stress (OS). Besides playing a role in cell differentiation, ROS could also influence mammographic density, another risk factor for breast cancer by activation of fibroblasts and thus secretion of collagen I. Our preliminary studies show that under acute ROS exposure, epithelial cells show loss of polarity and altered nuclear morphology that controls chromatin arrangement and differentiation. Our central hypothesis is that microenvironmental stress in synergy with altered matrix stiffness leads to increased breast cancer risk. To test our hypothesis we use a 3D cell culture model that comprises of non-neoplastic human mammary epithelial HMT-3522 S1 and human mammary fibroblasts HMS32-hTERT cells. Acute OS was induced by exposure to 250 μM H_2O_2 for four hours and chronic OS by treating the cells with 25 μM H_2O_2 for 10 days. Phenotypic changes in fibroblasts were assessed using immunostaining the nuclei for DAPI, and phalloidin staining for cytoskeleton. To test the effect of matrix stiffness on nuclear morphology S1 cells were cultured on collagen I coated with laminin 111 to induce differentiation, and fibroblasts were embedded in collagen I matrix of normal and at-risk stiffnesses *in vivo*. Fibroblasts show a loss of spindle phenotype under chronic ROS. Both chronic ROS and increased stiffness lead to less circular and bigger nuclei in S1 cells suggesting that both ROS and matrix stiffness impact epithelial cells and fibroblasts independently. Effect of increased stiffness and ROS are being investigated by coculture of S1 cells on top of fibroblasts embedded in collagen1 matrix.

1402 Interaction and Adaptative Response of HepG2 Cells Exposed to Low Concentration of Mixtures of T-2 and Its Modified Forms

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The T-2 toxin (T-2) is a mycotoxin belonging to type A trichothecene produced by *Fusarium* species. The T-2 is a contaminant of considerable concern due to its wide distribution and its highly toxic effects. The common metabolites of T-2 in human are HT-2 toxin (HT-2), neosolaniol (NEO), T2-triol and T2-tetraol. The aims of this study were to determine the cytotoxic effect of individual mycotoxins T-2 and its modified forms in human hepatocarcinoma (HepG2) cells; to evaluate the adaptative response of cells to adverse effects of T-2 and its modified forms, and to establish the interaction of combining multiple mycotoxins. Cytotoxic effects were determined in HepG2 cells by the MTT and total protein content (PT) assays after 24 and 48 hours of exposure. Concentrations used were: T-2 (5-100 nM), HT-2 and NEO (5-150 nM), T2-triol (160-2620 nM) and T2-tetraol (210-3350 nM). Results show that cytotoxicity increase depending on the concentration and exposure time. The IC_{50} for T-2 and its modified forms values were similar by both assays used. The IC_{50} range from 62 to 71 nM for T-2, from 60 to 68 nM for HT-2, from 56 to 121 nM for NEO, from 870 to 1240 nM for T2-triol and from 1380 to 2770 nM for T2-tetraol. To determine the hormesis effect, cells were pretreated with 2.5-10 nM of mycotoxin during 2 and 24 hours. Then, the medium was removed and increasing concentrations of each mycotoxin were added. The results obtained demonstrated that hormesis is an adaptative process in HepG2 cells as consequence of a favorable biological response only when low pretreatment of mycotoxins were used or at short time of exposure. To evaluate the interactions of mycotoxins mixtures, the isobologram analysis was applied for the five mycotoxins assayed in binary and tertiary combinations. The higher cytotoxic effect in mixture combinations compared to individual toxicity was observed. In the combinations, a synergistic effect was detected (CI < 0.75 ± 0.09) at low fraction affected (*fa*), whereas, at medium-high *fa* additive effects (CI from 1.05 ± 0.14 to 1.51 ± 0.31) were observed for the mixtures. In some combinations antagonistic effect (CI > 2.05 ± 0.37) was observed, and it could be associated to the competition of the mycotoxins to similar receptors. *Acknowledgements: Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R).*

1403 Effect of Variable Electronic Cigarette Voltage on Respiratory Cell Barrier Model

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The popularity of electronic cigarette has grown over time, whereas their health effects remain poorly explored. Traditional animal models are costly, ethically problematic, and do not suffice for the exploration of human toxic

exposures. The Calu-3 human derived adenocarcinoma cell line is a good compromise *in vitro* model. Calu-3 cell models are inexpensive, easily grown, and express important drug transporter proteins. When cultured at an air liquid interface (ALI), the monolayer polarizes and forms tight junctions and a functional respiratory epithelial barrier. Using a Calu-3 ALI model, we investigated the effects of varying e-cigarette aerosol generation voltage on the respiratory barrier as measured by Trans-Epithelial Electrical Resistance (TER). Calu-3 grown at ALI in trans-well units were exposed to an e-cigarette aerosol generated at 3.5V, 3.8V and 4.3V at a concentration of 10g/m^3 for 30 minutes after which their TER was recorded at 4, 24, and 48 hours post-exposure. We found an initial decrease in TER for all groups 4 hours post-exposure with increases over baseline values at 24 and 48 hours, particularly at the highest voltage. These findings may indicate instability in the cell model, barrier immaturity, or barrier stimulation following exposure to e-cigarette aerosols.

PS 1404 Novel Approaches to Predicting Bioeffects of Exposure to Unknown Compounds

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Rapid characterization and prediction of exposure bioeffects of unknown compounds is needed to improve military medicine, as the unique occupational environment contains myriad potential toxicants. Improved prediction methods are necessary to better inform decision makers and personnel of current or future exposure risks so that contact with potentially hazardous agents are minimized and mitigate overall health risks to our warfighters. This study assesses exposure risks of compounds that already exists in military environment - organophosphates and compounds in a USAF paint shop. A combinatorial approach - predictive computational modeling and *in vitro* studies - assessed toxicant effects in the lung. Thousands of compounds from the paint shop inventory was reduced to 53 representative compounds (volatile and non-volatile.) High-throughput toxicokinetics was used to estimate compound LC50s in cells, which provided midpoints in dose-titration treatment curves. A549 lung cells were treated with a dose-response curve of compounds for 24 or 48 hours followed by imaging. We assessed toxicity by cell viability and mitochondrial function. Images were ingested into our proprietary image analytics platform for unbiased image analysis, viability analysis and phenotyping. We quantified relative cell death from treatment of these compounds and, using unbiased phenotyping, uncovered morphological feature signatures that describe specific chemical exposures. We measured over 11,000 morphological features on every cell in each experiment - downselection of redundant features provide unbiased information about chemical exposure, as well as concentration/duration of exposure, allowing toxicological risk assessment. Comparative analyses allow determination of compounds that behave similarly and may share mechanisms of action, especially when compared with mechanistic toxicity assays. These feature fingerprints provide phenotype data to computational models, improving prediction of exposure. The results of this work improve predictive analytics efforts and enable creation of searchable databases containing *in vitro* toxicity and *in silico* pharmacokinetics, such that risk information can be extracted using *in vitro* image data or chemical structure.

PS 1405 Validation of Multiple *In Vitro* Skin Sensitization Tests

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Skin sensitization is the initial process that leads to an allergic response following contact with a chemical. The process by which skin sensitization occurs is outlined in an Adverse Outcome Pathway (AOP). The first Key Event (KE1) is the covalent binding of electrophilic substances to skin proteins. KE2 takes place in keratinocytes and the antioxidant/electrophile response element (ARE-Nrf2). KE3 is the activation of dendritic cells (DC). The OECD has published guidelines to test these AOP events using non-animal methods. We validated the following *in vitro* sensitization assays to provide alternatives to animal testing: Direct Peptide Reactivity Assay (DPRA; OECD 442C), KeratinoSens (OECD 442D), LuSens (OECD 442D); Human Cell Line Activation Test (h-CLAT; OECD 442E) and the Genomic Allergen Rapid Detection (GARD) skin assay. DPRA is used to measure KE1, KeratinoSens and LuSens for KE2, and h-CLAT and GARD skin for KE3, where h-CLAT measures increase in the expression of CD54 and CD86 surface markers, while GARDskin measures changes in RNA transcription. These assays can be used in a weight of evidence based approach (i.e. 2 out of 3 AOP events) to correctly identify skin sensitizing materials. We tested 10, 10, 10, 15 and 9 chemicals, in DPRA, KeratinoSens, LuSens, h-CLAT and GARD, respectively. In our laboratory, for these limited numbers of test chemicals, the DPRA achieved 100% accuracy, sensitivity and specificity. LuSens achieved 90% accuracy, 83% sensitivity and 100% specificity. KeratinoSens achieved 100% accuracy, sensitivity and specificity. h-CLAT achieved 100% accuracy, sensitivity and specificity. GARD achieved 100% accuracy, sensitivity and specificity. These results are evidence for the consistency and reliability of *in vitro* sensitization assays. By incorporating *in vitro* assays in the OECD framework for the testing of chemicals, dermal sensitization testing has the potential to move further away from the use of animal models and fulfill the longstanding aim of reducing, refining and replacing animal testing altogether.

PS 1406 Further Validation of a Nonanimal Integrated Testing Strategy (ITS) for Identification of Eye Irritants (GHS Category 2) Using the BCOP-EIT

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OECD non-animal test guidelines for identification of ocular hazards state no single *in vitro* test method will be able to fully replace the *in vivo* Draize eye test to predict across the full range of serious eye damage/eye irritation responses for different chemical classes. GHS guidance further states that assignment of GHS Category 2 (eye irritant) classification is impossible using any single test. Based on these recognized limitations, we have previously evaluated an integrated testing strategy (ITS) combining the EpiOcular™ Eye Irritation Test; EIT (OECD 492) and the Bovine Corneal Opacity and Permeability (BCOP) Test (OECD 437) to identify eye irritants (GHS Category 2). By using the combination of the EIT and BCOP test, we have accurately identified GHS Acute Eye Hazard Category 2 chemicals that cause reversible eye irritation. When a BCOP test rules out GHS Category 1 and the EIT rules out GHS No Category, analysis of these results indicates the only other possible designation - Category 2. Per GHS, Category 2 classification defaults to Category 2A, because differentiation between Category 2A and 2B cannot be made. After additional blinded testing, expanding our dataset from 42 to 51 chemicals, we correctly identified 92% of the Category 2A/B chemicals as Category 2. This additional testing raised our overall accuracy for predicting Category 1, Category 2, or No Category to 90%. This novel BCOP-EIT integrated testing strategy has been adopted by several chemical manufacturers to identify ocular hazards while simultaneously reducing animal usage.

PS 1407 A Mathematical Model for Estimating the Dissolution Rate of Synthetic Vitreous Fibers from *In Vivo* Studies

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Currently, measurement of biopersistence of synthetic vitreous fibers (SVF's) uses rat models to demonstrate regulatory compliance, typically via the half time ($T_{1/2}$) for clearance of long, respirable fibers. Historically the clearance rate of long fibers in such tests was associated with the rate of dissolution (K_{dis}) of fibers in near-neutral pH simulated lung fluids. *In vitro* testing mimicked the interaction between an inhaled fiber and extracellular fluid in the pleural space, which resulted in predictive models based on fiber dissolution rates at near-neutral pH. Developments in SVF chemistry resulted in fibers which demonstrated low biopersistence despite very low solubility at neutral pH, challenging the 'single dissolution rate' models. Clearance of these fibers is often mimicked using a dissolution rate at acidic pH (K_{acid}). Here we address this complexity with a single model which can estimate the clearance rates of commercial SVF's. We based the model on *in vivo* fiber clearance data with the outcome a prediction of long, short, and WHO fiber clearance over time. The model uses an initial distribution of fibers (length and diameter) and follows the time evolution of the fiber length-diameter distribution. Iterative simulations incrementally varying K_{dis} and K_{acid} as the fitting parameters for each fiber while minimizing the chi-squared statistic let us determine the 'dissolution rates' showing the best agreement with experimental data .A series of simulations using publicly available *in vivo* clearance was used to refine the initial concept into a working simulation methodology. The methodology that best described known *in vivo* results involves following the time evolution of an initial distribution of fibers subject to three potential clearance mechanisms (1) long fiber cleavage by acidic dissolution (macrophage attack) (k_{acid}) (2) macrophage mediated clearance of short (< 15 micron length) fibers and (3) congruent dissolution of the fibers at a neutral pH (k_{dis}). Studying the relationship between the tested fiber chemistry and these 'best estimates' of dissolution rate permitted the development of correlations between fiber chemistry and K_{dis} and K_{acid} which provide useful design guidance. Going forward, beyond the value for fiber developers, the authors believe this ap-

proach and/or improvements on this approach could become a viable tool for avoiding animal studies when assessing the biopersistence of glass, stone, and slag wool fibers as required by Note Q of Regulation (EC) No 1272/2008.

1408 Inflammatory Profile of Respiratory System Cells as an *In Vitro* Tool for Evaluation of Respiratory Sensitizers

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Inhalation toxicity is traditionally evaluated using rodents, which can be submitted to different exposure schemes for assessment of acute, sub-acute and sub-chronic toxicity of a test material. However, although such *in vivo* models are the only that have regulatory acceptance, these models only employ the lethality as an endpoint for classification, while effects upon the respiratory system are not evaluated separately. Moreover, there are no available methods for evaluation of pulmonary allergenicity and this is partially due to the fact that the Adverse Outcome Pathway (AOP) for lung sensitization remains incompletely understood. The objective of this work was to evaluate the inflammatory alterations triggered by different respiratory sensitizers upon different cell types of the respiratory tract. We selected six respiratory allergens (Chloramine-T, Piperazine, Maleic Anhydride, Glutaraldehyde, Cyanuric Chloride and Trimetilic Anhydride Chloride) and evaluated the cytotoxicity upon four different cell types, including bronchial epithelial cells (BEAS-2B), lung fibroblasts (MRC-5), endothelial cells (EA.hy926) and monocytic cells (THP-1), using the MTT reduction assay. Further, we exposed these cells to the cell viability 80% concentration of each test material for 24 hours, prior to determination of pro-inflammatory (IL-1β, IL-6, IL-8, IL-12p70 and TNF-α) and anti-inflammatory (IL-10) cytokines levels, using the Cytometric Bead Array (CBA) method in both cell lysates and supernatants. The results demonstrated that respiratory sensitizers promoted inflammatory alterations in all the investigated cell types, increasing the production of pro-inflammatory cytokines, highlighting the involvement of epithelial and monocytic cells. Besides that, all the cells increased the production of IL-8 in response to exposure. The obtained results demonstrate that the production of pro-inflammatory cytokines by respiratory system cells can be a valuable endpoint for *in vitro* assessment of lung sensitization potential. Furthermore, the production of IL-8 by such cells seems to be a determinant feature for the chemically induced respiratory disorders.

1409 Utility of the VITROCELL Cloud System for Investigating the Effect of Oseltamivir on Human Airway Epithelium *In Vitro* after H1N1 Infection

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Respiratory viral infections, such as flu, are a worldwide concern and a source of huge economic burden for the society. Most antiviral drugs to date are delivered systemically by an oral or intravenous route of administration. Inhalation of aerosolized antiviral drugs would not only allow direct and quick access to the site of infection but also help avoid first-pass effects. As such, aerosolized drug delivery could increase the antiviral efficiency of drugs while reducing their side effects. To explore the *in vitro* efficiency/toxicity of an antiviral drug (prescribed for oral treatment in humans), we describe here a novel method for delivering oseltamivir to the infection site to counter a virus infection (H1N1 virus) through aerosolization. To this end, we took advantage of the VITROCELL™ Cloud system to nebulize oseltamivir and expose 3D organotypic air-liquid interface (ALI) tissue cultures to the drug. In contrast to more complex exposure systems that are based on continuous flow, the VITROCELL™ Cloud system operates through an injection mode of aerosol delivery by using a clinically relevant nebulizer, which also limits the need for larger quantities of the drug for testing. MucilAir™Pool, an *in vitro* human reconstituted standardized nasal epithelia pooled from 14 different donors, is a physiologically relevant test system for studying antiviral compounds. By using this system in combination with the "Cloud chamber", we were able to successfully demonstrate the efficiency of nebulized oseltamivir against H1N1 infection as well as preservation of respiratory epithelial function, with low cytotoxicity (less than 20%). The efficiency of oseltamivir has been demonstrated by a decrease in viral copy number as well as through endpoints responsive to viral infection, such as ciliary motility and secretion of soluble factors (RANTES, CAMP, and β-defensin 2). Overall, the results of this conceptual study highlight the combination of VITROCELL™ Cloud system and MucilAir™ as a suitable *in vitro* exposure and test system for inhalable drugs as well as inhalation as an alternative to oral delivery of antivirals.

1410 Alternative Testing Strategies for Assessing Eye Irritation/Corrosion of Agrochemicals under the New Brazilian Human Health Authority (ANVISA) Regulation

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Global harmonization in toxicological testing requirements is a need to implementation of alternative testing strategies for agrochemical formulations. The recent Brazilian regulation for human health toxicological assessment of pesticides (ANVISA Res. 294, 2019) established scientific weight of evidence (WoE) and harmonized approaches to international best practices including application of GHS for classification and labeling for end-use products, risk-based assessment of new active ingredients and promotion of new assessment methodologies. Historically, accepted regulatory tests for formulated products have generally been *in vivo*. Alternative methods has been legally introduced in the new resolution. However, there is a challenge to better define their applicability domain and the uncertainties related to their prediction of human outcomes. Eye irritation has generally been performed in Albino rabbits according to the Draize method (OECD 405). This poster presents a tiered approach for assessment of eye irritation/corrosion endpoints for regulatory evaluation of agrochemical formulations in Brazil using example case-studies. First, evaluation of the necessity of testing and a decision between top-down vs bottom-up approach is based on existing information including pH (≤2or ≥11.5; avoid test and self-classify as corrosive) and outcome of the GHS calculation. If the test item is evaluated as corrosive, the top-down approach is selected, and if irritating or non-irritating (NC), a bottom-up approach is recommended. For example, if the bottom-up approach is selected, EpiOcular test (OECD 492) is conducted and if negative results are obtained, no further testing is necessary; otherwise a corrosion test as Bovine Corneal Opacity and Permeability (OECD 437) should be performed. Based on calculation methods, *in vitro* results and prevalence of irritancy properties for the product class of agrochemical formulations, the test item may be classified as GHS categories 1 or 2 or NC. An *in vivo* test should be used as a last resort and may clarify the severity and persistency of ocular damage, where required. Regardless of the testing strategy selected, relevant information should always be presented as a WoE taking into consideration the respective strengths and limitations of each method used.

1411 New Approach to Evaluation and Classification of Irritation Intensity of Oral Care Ingredients Using a Three-Dimensional Human Buccal Mucosal Model

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Oral mucosal irritation is an important issue in the safety assessment of oral care products and ingredients. A three-dimensional human buccal mucosal model has been established as an alternative to the oral mucosal irritation test *in vitro, and the new EpiOral (manufactured by MatTek Corp., Ashland, MA, USA)* is one such model. Here, investigators at Kao Corporation, Sunstar Inc. and LION Corporation aimed to determine whether the EpiOral could serve as an *in vitro* test that reflects oral mucosal irritation scores *in vivo* by evaluating the concentration dependence, inter-facility reproducibility, and correlations between *in vivo* and *in vitro* findings based on cell viability. Five materials for oral care products that have been evaluated in terms of irritation *in vivo* according to Cosmetic, Toiletry, and Fragrance Association (CTFA) guidelines served as test materials. Cells that had been incubated with cultured EpiOral for 30 min were exposed to 100 µL of each test material (0.25%, 1%, 2%, 4%) for 30 or 120 minutes. Thereafter, the test material was rinsed out with PBS, and cell viability was calculated using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assays. Cell viability significantly correlated with oral mucosal irritation scores *in vivo* and the duration of exposure to each test material (ANOVA, $P < 0.0001$). Inter- and intra-facility reproducibility and concentration dependence were confirmed. These data indicated that materials with irritation scores *in vivo* of ≥ 1 could be distinguished from non-irritating materials based on 60% cell viability at 120 minutes of exposure. We also found that materials with irritation scores *in vivo* of > 3, could be distinguished from those with irritation scores *in vivo* of 1 to ≤ 3 based on 60% cell viability at 30 minutes of exposure. These results support a new approach to establishing an oral mucosal irritation test *in vitro* using different exposure durations.

1412 Evaluation of Historic *In Vivo* Data to Characterize the Emetic Properties of Liquid Cleaning Products and Provide a Framework for the Development of an *In Silico* Predictive Algorithm

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Accidental ingestion of household cleaning products frequently results in emesis (vomiting) in both children and adults but it is not known which physicochemical properties of the formulation are primarily responsible. Preclinical studies using dogs to assess the emetic potential of product formulations administered by gavage were undertaken routinely by industry over 30 years ago but this is no longer the case. To investigate whether the data on emesis collected in these extensive historic company-sponsored studies could provide novel insights into the constituents responsible for emesis, we re-analysed the historic data for a total of 74 liquid cleaning formulations of which 6.7% did not evoke emesis within 60 min. The incidence (Respond/Tested) of emesis was dose-related with calculated ED_{50} values between 0.012-8.4 ml/kg with 57% of formulations having an $ED_{50} \leq 1$ ml/kg. The median value for the latency of onset of emesis was 10.0 min (95% CI, 8-12 min) with the number of vomits over one hour ranging from 1-10 (median 2). Detailed analysis of all components (e.g., incidence, latency and intensity) of the emetic response for a subset of formulations and their physicochemical properties revealed that formulations with a high percentage of non-ionic surfactants (alcohol ethoxylates) or high ionic strength were associated with a higher emetic potential. This was independently verified by statistical modeling, i.e., multivariable linear regression and recursive partitioning analysis, using calculated ED_{50} values from these historical beagle dog studies and formula ingredients/physiochemical measures as variables. By comparing the present data with published studies of a range of emetic agents administered by gavage in the dog and mechanistic studies in other species, we hypothesise a critical role for the enteroendocrine cells in the mucosa of the upper digestive tract. Although using historic data collected over an extended period has limitations (e.g. methodological changes), analysis of such data has provided novel insights into the emetic characteristics of this class of product and has informed the development of a *in silico* model to predict the emetic liability of novel formulations without the need for additional *in vivo* studies, hence contributing to 3Rs principles.

1413 Development of an *In Vitro* Exposure-Circulation-Metabolism Model to Mimic the State of Nicotine Exposure to the Lung and Metabolism by the Liver

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Single-cell cultures have a strong advantage in studying specific endpoints, but they show insufficient ability to predict and assess the toxic effects of exogenous substances after metabolic activation *in vivo*. When microfluidic and co-culture techniques are widely used for *in vitro* toxicity assessment, this study aims to establish an *in vitro* nicotine exposure-circulation-metabolism model (ECMM), namely: a model that simulates the circulation of nicotine through the lungs into the blood vessels of the body to the liver, and the metabolites are exposed to the lungs. In this study, an exposure-circulation-metabolism platform (ECMP) was constructed by selecting suitable materials, namely, tube (D: macrophages), circulating pump, and three independent chambers (A: Beas-2b; B: Huvec; C: 25 donor liver microsomes) were connected in series to form a loop. In the platform, the morphology of Beas-2b and macrophages were observed under the microscope to determine whether the platform was suitable for cell growth; CCK-8 and ELISA were used to detect the cell viability and IL-8 expression levels of macrophages at different flow rates and times to obtain a flow rate that did not cause cell damage and inflammation; the distribution of the loading buffer was used to determine whether the platform allowed material transfer. Microscope and CCK-8 were used to detect the cell morphology and cell proliferation curve of Beas-2b, Huvec and macrophages in different medium ratios and cell concentrations to obtain optimal culture conditions. The cells and liver microsomes were cultured in the platform under optimized conditions to establish an ECMM. In this model, nicotine was added to the chamber A, and nicotine and cotinine in chamber A were detected by HPLC-MS/MS 3h later. Beas-2b and macrophages could be well grown in ECMP constructed by transwell chambers, glass, rubber tubes, dialysis bags and 0-5 ml/min circulating pumps, etc.; the flow rate of 1 mL/min had no significant effect on macrophages damage and inflammatory reaction; and the loading buffer could be fully delivered in the platform within 2h at 1 mL/min; under the culture conditions (Medium:BEBM: ECM: DMEM (1:1:1; 500 mL) + respective factors + serum 25ml + $5*10^4$cells/ml+1ml/min), the three cells could proliferate well, substance could be passed and liver microsomes could exercise metabolic function; within this model, chamber A nicotine was significantly reduced and cotinine production was found. These results indicate that this study established a nicotine ECMM *in vitro* by using Beas-2b, Huvec, 25 donor liver microsomes and macrophages, which is expected to be used for *in vitro* exposure metabolic toxicology test.

1414 Characterization and Use of Organotypic Lung Model to Assess the Pulmonary Fibrosis of Nanomaterials

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Inhalation is one of the major routes by which exposure to nanomaterials (such as multi-walled carbon nanotubes (MWCNTs)) may occur and lead to adverse effects in humans, such as pulmonary fibrosis. Presented here is a reconstructed primary human alveolar cell-based model (EpiAlveolar™, MatTek Corp.) that can be used to assess the potential of test materials to cause pulmonary fibrosis. EpiAlveolar™ —comprised of alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts—was developed to mimic the *in vivo* human alveolar microenvironment. Characterization of the model shows presence of alveolar epithelial Type I (ATI) and Type II (ATII) cells, expression of pro-surfactant C, and formation of a tight epithelial barrier. When challenged with transforming growth factor β (TGF-β)—a known positive control for pulmonary fibrosis—the EpiAlveolar™ model demonstrated gradual signs of fibrosis over a period of 21 days such as a significant decrease in barrier integrity (transepithelial electrical resistance (TEER)), increase in extra-cellular matrix proteins (fibronectin and collagen 1A1), and thickening of tissues (hematoxylin and eosin staining). The aforementioned fibrotic biomarkers were also expressed after repeated sub-chronic exposures (1 - 30 μg/cm^2 for up to 21 days) to two types of MWCNTs (Mitsui-7 and Nanocyl) using an air-liquid interface exposure device (VITROCELL® Cloud). The EpiAlveolar™ model has shown value in measuring several key events along the proposed adverse outcome pathway for pulmonary fibrosis (https://aopwiki.org/aops/173). This system can be used in a mechanism-based *in vitro* testing strategy using human-relevant methods to predict pulmonary toxicity and to enable effective risk assessment of substances including MWCNTs.

1415 Variability in the Rabbit Skin Irritation Assay

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For decades, the gold standard for skin irritation testing has been the *in vivo* rabbit skin test. As a result, it has been the benchmark against which new approach methodologies have been compared. *In vitro* methods following the Organisation for Economic Co-operation and Development's Test Guideline 439 are accepted as partial replacements for the *in vivo* test because they are capable of separating irritant from non-irritant chemicals. However, because none are capable of classifying moderate and mild irritants, there are currently no *in vitro* methods that are considered full replacements for the *in vivo* assay. A limiting factor in identifying a full replacement for the *in vivo* method could be the variability inherent to the subjective scoring of erythema and edema responses in the rabbit test. This is particularly relevant for mild and moderate irritants, which alternative methods have had difficulty differentiating with high concordance. To better characterize the reproducibility of the *in vivo* assay, in this study we assessed variability in results from chemicals tested multiple times. We compiled and curated a dataset of 3056 test records, representing 727 unique chemicals for which GHS classifications were available. Each chemical was tested at least twice in the *in vivo* assay. Where possible, primary dermal irritation indexes (PDII) were estimated from the available data and used to classify chemicals according to the US Environmental Protection Agency skin irritation classification criteria. Conditional probabilities were used to evaluate the reproducibility of the *in vivo* method in identification of severe, moderate, and mild irritants, and non-irritants, according to both GHS and EPA categorization methods. Chemicals classified as moderate irritants at least once were classified as mild irritants or non-irritants over 40% of the time when tested repeatedly. The level of variability found was greatest between mild and moderate irritants. This analysis indicates that the level of variability present in the rabbit skin irritation test should be taken into consideration when evaluating the performance of nonanimal alternative methods.
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1416 Examining the Effects of Volatile Agents Using Cell-Based Gaseous Metabolite Production

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Many agents (e.g., pollutants, pharmaceuticals, additives) requiring toxicity testing are gaseous and cannot be screened adequately in standard cell exposure systems. Submerged cell culture systems impede delivery of gases to cells due to media interference. Gas-media component reactions could form toxic products. We are using an exposure system and cell line (BEAS-2B human airway epithelial cell line) that can tolerate a lack of medium on the apical cell side for potential screening of gaseous agents minimizing the confounding effects of media. The system also can examine the conversion of an exogenously added volatile substrate to a gaseous metabolite. Monitoring production of a gas phase metabolite can be utilized to screen metabolism perturbations induced by an agent of interest (i.e., "probe molecule approach" to toxicity testing). Cells were incubated with 1 or 2% ethanol (EtOH) for up to 6 hr without the loss of cell viability (using released LDH activity and trypan blue dye exclusion). In a 37ºC flow through system (with air plus 5% CO_2 inflow), EtOH was added apart from the cell culture dishes. Acetaldehyde (C2) was collected onto dinitrophenylhydrazine packed cartridges on the outflow line. C2 was eluted from the cartridges and analyzed by LC\UV\MS. With EtOH exposures alone, increased cell C2 production was observed compared to vehicle controls (0% EtOH). The effects of pharmaceutical and flavoring agents on EtOH-induced C2 production were then examined. Cells pretreated with disulfiram (to inhibit C2 catabolism) and exposed to EtOH had increased C2 compared to untreated cells. Co-incubation of cells with EtOH and an e-cigarette flavoring component, cinnamaldehyde, decreased C2 production suggesting effects on alcohol dehydrogenase and/or other enzymes. These findings show the utility of this exposure system for measuring effects of different classes of agents on the metabolism by an important enzyme pathway. The use of this flow through gas system shows promise for 1) the ability to expose cells to volatile substances and preserve cell viability for several hours, and 2) capture a gas phase metabolite for use in probe molecule approaches for screening the induction of toxicity by gaseous agents. The use of *in vitro* substrate metabolism for chemical toxicity screening may reflect *in vivo* metabolism responses induced by exogenous chemicals. *This abstract may not reflect official US EPA policy.*

1417 Using a Measurement Science Approach to Increase Confidence in the EASA Skin Sensitization Assay

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NICEATM is coordinating a multi-laboratory validation study to characterize the Electrophilic Allergen Screening Assay (EASA) for risk and hazard assessment for skin sensitization classification. The EASA assay functions by assessing potential skin sensitizers by measuring depletion of one of two probe molecules. The use of this assay in a single cuvette format resulted in multiple measurement challenges, such as low throughput and inability to include sufficient control measurements. We redesigned this assay protocol to work with a 96-well plate format. Process control measurements were incorporated into a 96-well plate design to quantify key sources of variability each time the assay is run. Examples of process controls added are: positive control standard curve, bubble/precipitate interference, and QC charting. We also conducted considerable robustness testing (i.e. stability, pipetting, disposables, etc.) Additional control experiments were performed to evaluate photodegradation of probe molecules, bias from bubbles caused by pipetting, and statistical power of the assay system. Sixty compounds from NTP were tested in the 96-well plate design. One key insight revealed by this process was the interference from test compounds, namely producing an absorbance or fluorescence signal similar to that of the probe molecules, which would not have been previously detected using the single cuvette assay. Not taking the interference into account has been shown to lead to potential false negative identification. We also replaced the traditional static call line (i.e. 3 x SD) with a modified t-test to perform custom tailored "call lines" for each compound tested taking into account the uncertainty around its measurements. The data from the 60 compounds were analyzed using a Bayesian analysis as well as the student t-test statistic and has approximately 70 - 75% concordance with LLNA data. Overall, the measurement science approach described here provides steps that can be taken to increase confidence for *in vitro* assays by fully characterizing sources of variability and potential biases in the assay that will facilitate interlaboratory testing and standardization.

1418 A Novel Strategy Combining Kao ITS and Read-Across to Determine the Skin Sensitization Potency of Cosmetic Ingredients

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The integrated testing strategy (ITS) developed by Kao covers three alternative models, namely h-CLAT, DPRA and DEREK, as a defined approach for the potency classification (EC3 < 2% = Strong; EC3 ≥ 2% = Weak; Non-sensitizer) of skin sensitization. To enable a quantitative risk assessment, especially for weak sensitizers, it is important to refine the potency estimation and to determine a specific point of departure (PoD). To address this issue, we developed a strategy combining the ITS and read-across using relevant analogues with LLNA data. Firstly, the ITS for a target chemical was performed to classify the sensitizing potency. The confidence in the ITS was evaluated based on predictive limitations. In a second step, read-across was performed to identify suitable analogues of the target, using four *in silico* tools (ToxGPS, ToxRead, OECD TB, TIMES-SS). By comparing the analogue LLNA data with the ITS result, the level of confidence in the ITS outcome was increased and a specific PoD was derived. To determine the applicability of the novel strategy, we performed two case studies, using weakly sensitizing cosmetic ingredients with known EC3 values, namely HC Blue No. 12 (EC3 = 5.0%) and 2,4-Diaminophenoxyethanol HCl (EC3 = 3.2%). The ITS correctly predicted both case study chemicals as EC3 ≥ 2%, indicating a high level of confidence in the classification. The identified analogue for HC Blue No. 12, namely HC Blue No. 2, showed a PoD (EC3 = 4.1%) that was close to the true EC3 of the target. Based on a comprehensive weight of evidence, the identified analogue EC3 value could serve as a relevant PoD for the target. The EC3 of 14.7% of 1,3-Bis-(2,4-Diaminophenoxy) Propane HCl, the identified analogue of Diaminophenoxyethanol HCl, was overpredicted by the ITS with an EC3 < 2%. Moreover, the results obtained from the *in vitro* tests and *in silico* predictions were inconsistent for the case study chemical and its analogue. These uncertainties led us to the conclusion that a lower limit of EC3 = 2% could serve as relevant target PoD. This value is more conservative than the true EC3 of the target. In summary, our case studies indicate that our novel strategy could be useful to estimate the skin sensitization potency of cosmetic ingredients sufficiently conservative.

1419 Development of QSAR Modeling for hERG Blockers and Protectors Using Large Datasets

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hERG (human ether-a-go-go related gene) potassium channel is blocked by numerous structurally and functionally unrelated drugs, leading to life-threatening arrhythmias and cardiac death. It also leads to prolonged QT intervals. This made drugs of diverse chemical structures to be withdrawn from the market. Having a good prediction model with high coverage and accuracy would help in accurate identification of hERG blockers. Currently there are multiple QSAR models on hERG inhibition, each with a different approach. Our set of hERG models include hERG Blockers which helps in prediction of chemicals that block the potassium channel thereby causing long QT intervals and hERG Protectors which predicts if the chemical diminishes the potassium channel block and protects against long QT syndrome. The datasets are collected from various research papers and pubchem assays. The dataset for hERG Blocker started with 306895 (8723 positives, 298172 negatives) records and ended with a 12537 (5769 positives, 6768 negatives) training set and 3495 external set records. Similarly, hERG protectors started 305679 (1552 positives, 304127 negatives) records and ended with 2381 (1206 positives, 1175 negatives) training set and 622 external set records. Even though the dataset is reduced in its size because of smaller number of actives and curation of salts and mixtures, functionality of all the excluded chemicals are covered during the model building process by the addition of extra chemicals causing "out of domain" calls. These models can identify most of the common drugs withdrawn from the market accurately i.e. Cisapride, Sertindole, Terfenadine, Astemizole etc. The results for external validations of hERG Blockers model are 93% sensitivity, 93% specificity, 92% positive accuracy, 94% negative accuracy, 94% coverage, 0.954 ROC. With bootstrap cross validations, model exhibited 92% sensitivity, 90% specificity, 89% positive accuracy, 93% negative accuracy, 93% coverage, 0.942 AUC. The results for external validations of hERG Protectors model are 71% sensitivity, 71% specificity, 72% positive accuracy, 69% negative accuracy, 78% coverage, 0.726 ROC. With bootstrap cross validations, model exhibited 65% sensitivity, 68% specificity, 70% positive accuracy, 63% negative accuracy, 70% coverage, 0.669 AUC. This predictivity is higher than many of the currently available hERG models. Thus, we successfully built highly interpretable and accurate QSAR model for hERG.

1420 *In Vitro* Buccal Membrane Absorption Model for Tobacco Constituent Testing: Membrane Integrity Assay Assessment

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Assays to provide information on chemical hazards and the influence of harmful and potentially harmful constituents (HPHCs) of tobacco on buccal permeability and absorption via the buccal pathway are sparse in the literature. Therefore, an *in vitro* buccal membrane absorption model (IVBMAM) was developed using porcine buccal mucosa as a tissue surrogate for human buccal mucosa given similarities in morphology and permeability. The model was developed by adapting *in vitro* dermal absorption methodologies. Membrane integrity assays were compared for use in validating test tissues. Porcine buccal mucosa was isolated from connective tissue and cut with a dermatome to a thickness of 400-500 µm. Buccal mucosa was mounted in flow-through diffusion cells and saliva permeability coefficients (Kp) were measured using tritiated artificial saliva, pH 7.7. The average baseline Kp value for porcine buccal mucosa using tritiated saliva was 2.5×10^{-2} cm/h (n = 6). The average baseline Kp value for porcine and human skin using tritiated saliva was 3.7×10^{-3} cm/h (n = 3) and 2.7×10^{-3} cm/h (n=3), respectively. Saliva Kp values were compared to transepidermal water loss (TEWL) and transepithelial electrical resistance (TEER) measurements to determine baseline criteria for the IVBMAM and dermal tissues. Buccal mucosa mounted on transwell plates exhibited average normalized TEER levels of 114.7 Ωcm^2, compared to 78.4 Ωcm^2 for porcine skin and 78.6 Ωcm^2 for human skin. TEWL values for the buccal mucosa averaged 67.3 $g/m^2/h$, compared to 40.9 and 6.1 $g/m^2/h$ for porcine and human skin, respectively. Saliva Kp values were over 10-times higher in buccal mucosa compared to skin (porcine and human). TEWL values were over 1.5-times higher in buccal mucosa compared to porcine skin and 10-times higher than human skin. TEER values for the buccal mucosa were about 1.5-times higher than the skin values. TEER values did not correlate with membrane integrity as measured by Kp and TEWL. Therefore, TEWL may be a better non-radiolabled measure of membrane integrity than TEER for this IVBMAM. *Supported in part by Oak Ridge Institute for Science and Education. Disclaimer: This presentation is not a formal dissemination of information by US FDA and does not represent Agency position or policy.*

1421 The Utility of a Human *In Vitro* Population-Based Model for Studies of Epigenetic and Genotoxic Mechanisms

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Genetic variability has a major impact on susceptibility to chemical exposures, yet there are few experimental models that address inter-individual variability, a critical need in hazard and risk assessment. We have previously demonstrated inter-individual variability in epigenetic effects and genotoxicity using a mouse population approach and a model known human carcinogen, 1,3-butadiene. Although data from an *in vivo* mouse model are important, they require animal experiments and species extrapolation. In this study, we tested a human *in vitro* population-based model as an experimental approach to assess and quantify variability in epigenetic alterations and genotoxicity in response to 1,3-butadiene metabolites. Specifically, we tested the hypothesis that a population-based model of human lymphoblasts and human pluripotent stem cells from a diverse set of individuals can be used to evaluate inter-individual variability in epigenetic and genotoxic effects of chemicals. We conducted a concentration-response screening of three butadiene metabolites (1,2-epoxybutene, 1,2,3,4-diepoxybutane and 3,4-epoxy-1,2-butanediol) in panels of human lymphoblasts and pluripotent stem cells from 100 and 30 individuals, respectively, that also included both sexes. Lymphoblast lines were from the 1000 Genomes Project and represented 9 populations of European, African, Asian, and admixed ancestry. The pluripotent stem cells were from the California Institute for Regenerative Medicine Induced Pluripotent Stem Cell Repository and represented 4 populations of African, Asian, Hispanic, and Caucasian. We evaluated 1,3-butadiene-induced cytotoxicity by measuring cell viability. Population variability in response to 1,2,3,4-diepoxybutane was most pronounced. However, there was minimal variability in the effects of 1,2-epoxybutene and 3,4-epoxy-1,2-butanediol. Next, we selected 10 pluripotent stem cells and 10 lymphoblast cell lines based on the variability in responses to cytotoxicity. Genotoxicity was evaluated by measuring N-7-(2,3,4-trihydroxybut-1-yl)-guanine adducts. For assessment of the changes in chromatin states, we used the assay for transposase accessible chromatin (ATAC) followed by sequencing. In addition, transcriptomic changes were assessed by using Tempo-seq. Overall this work evaluates the use of a novel human *in vitro* population-based model, which could serve as a valuable tool for high-throughput chemical screening to assess human risks of chemical exposure.

1422 Inter- and Intra-Laboratory Reproducibility of a Three-Dimensional Model of Human Buccal Tissue

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Human 3D tissue models of the human oral epithelia have been developed as alternatives to animal testing. These models are powerful tools for investigating basic biological function in response to various challenges including oral care products and environmental insults. To evaluate inter-laboratory reproducibility of the commercially available 3D buccal epithelial model, EpiOral, MTT tissue viability was monitored. Tissues were shipped to Lion Corp (Idawara, Japan) and Kurabo Industries (Osaka, Japan) and the MTT values for negative control (NC) tissues (exposed to ultrapure water, 60 min) and the time to reduce tissue viability to 50% (ET-50), following exposure to 1% Triton X-100, were determined. NC tissues gave an average MTT value of 1.633 (n=15 lots) and 1.829 (n=11) at Lion and Kurabo, respectively, in independent tissue lots; tissues packaged and stored overnight at MatTek gave NC values of 1.545 (n=22 lots). ET-50 of tissues tested at Lion and Kurabo averaged 64.3 ± 12.9 (n=15) and 54.7 ± 10.3 (n=11) min, respectively; tissues tested at MatTek gave 79.6 ± 13.7 min (n=22). All lots tested in Japan and the US fell within the acceptance QC range for the NC (MTT > 1.0) and ET-50 (34.8-105.8 min). In addition, histology of non-cornified buccal tissue produced in 2019 was highly reproducible (n=22 lots). For intra-laboratory reproducibility, functional analysis was performed by exposing the tissues to two inducers in independently produced tissue lots (n=3). Tissues were exposed to TNF-α/IL-1β to induce an inflammatory response or to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) to evaluate xenobiotic metabolism. Adenylate kinase release, MTT activity, pro-inflammatory cytokine release and xenobiotic phase I enzyme expression were analyzed. Exposure of the oral tissues to the inflammatory inducers, TNF-α/IL-1β, showed statistically significant increases in secreted IL-8, IP-10, and MMP-1. TCDD induction also showed increased gene expression of the Phase I metabolism enzymes, CYP1A1 and CYP1B1. Overall, excellent reproducibility was observed for MTT and adenylate kinase endpoints over n=3 independent lots. In addition, reproducible trends in cytokine release and gene expression versus the NC tissues were observed in the n=3 tissue lots. In summary, the EpiOral model exhibited good inter-lab and intra-lab reproducibility and can serve as a tool to reduce animal experimentation for oral toxicity studies.

1423 Acute Oral Toxicity *In Silico* Models: Performance on Pharmaceutical Compounds

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Acute oral toxicity (AOT) is generally defined by an LD50 which represents median lethal dose expected to cause death in 50% of animals (mg/kg) after an acute exposure. AOT hazards are generally conveyed on safety data sheets via GHS categories (United Nations Globally Harmonized System of Classification and Labeling of Chemicals), which are assigned based on the LD50 value as follows: Category 1: LD50 ≤5mg/kg; Category 2: 5<- ≤50mg/kg; Category 3: 50<-≤300mg/kg; Category 4: 300<-≤2000mg/kg; Category 5: 2000<-≤5000mg/kg. *In vivo* AOT studies are required by regulatory authorities with limited alternatives being accepted. AOT *in silico* models have been trained and validated utilizing publicly available data on common chemicals, which tend to be less complex PCs (pharmaceutical intermediates and APIs). Two AOT *in silico* models were evaluated within the PC space utilizing historical *in vivo* AOT data for >350 PCs. The *in silico* programs evaluated included the Leadscope AOT Suite (Leadscope) and the Collaborative Acute Toxicity Modeling Suite (CATMoS). Leadscope employs both an expert-alerts model and a statistical-based model to predict GHS AOT categories. Statistical-based models are generally referred to as Quantitative Structure-Activity Relationship (QSAR) models which predict toxicity endpoints using the chemical structure. Expert-rule-based platforms utilize expert knowledge of the structure to create rules around the likelihood of the compound to evoke toxicity. CATMoS has a QSAR prediction tool for five endpoints: Very-Toxic (LD50<50 mg/kg), Non-Toxic (LD50>2000 mg/kg), EPA Categories, GHS Categories, and LD50 point estimates. Structural files for >350 PCs were obtained and input into each *in silico* model and the AOT predictions were compared to historical data for each PC. It was found that 95% (365/384) of PCs fell within the CATMoS applicability domain and 88% (338/384) of the PCs fell within the Leadscope applicability domain (99% were covered by both mod-

els). The best overall accuracy was similar in the Leadscope statistical model (45%) and the CATMoS GHS Model (46%). Both programs exhibited low accuracy for the most hazardous compounds with the CATMoS VT model also showing 0% accuracy. The preferred model for worker safety purposes was Leadscope which accurately or over-predicted the GHS category for >90% (303/338) of compounds in domain. CATMoS, had a higher under-prediction rate of 28% (GHS model) and 18% (LD50 model).

1424 Generation of Novel *In Chemico/In Vitro* Skin Sensitization Data to Evaluate the Human Relevance of Defined Approaches

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Skin sensitisation, leading to allergic contact dermatitis, is a common occupational health problem. Consequently, chemicals in products intended for human use must be assessed for their skin sensitisation potential. Traditionally this assessment has been carried out using *in vivo* assays like the murine local lymph node assay (LLNA), however, political and ethical pressure has led to increased use of non-animal alternatives such as *in chemico* and *in vitro* assays. Results from multiple information sources (non-animal assays, *in silico* models, physicochemical parameters) are often combined in what is known as a defined approach (DA). It can be difficult to assess how well DAs predict human skin sensitisation potential as the amount of publicly available *in chemico/in vitro* data with corresponding human data is sparse. As such, a collaborative project was devised whereby chemicals lacking *in chemico/in vitro* data but with human potency data would be identified and new *in chemico/in vitro* data generated - ideally to test the relationship between *in chemico/in vitro* data and human potency. Human data were taken from two publications which assigned a human potency category between 1 (extreme sensitiser) and 6 (non-sensitiser) to mainly fragrance-like chemicals based on human repeat insult patch test data, clinical data, and exposure. 80 chemicals were found with no or limited *in chemico/in vitro* data and of these, 34 were commercially available at a reasonable cost. These 34 were then prioritised into three groups based on the availability of LLNA data and (lack of) *in chemico/in vitro* data. The first group consisted of 8 chemicals and so far during this collaboration, DPRA, KeratinoSens™, and h-CLAT data have been generated using blind testing for this group. The predictivity of the individual assays when compared against LLNA data was between 50% - 88% and between 38% - 75% when compared against human data. When these novel data are used within 3 published DAs, the BASF 2/3, Kao ITS, and Lhasa DA, the predictivity against *in vivo* data ranges from 38% - 88%.

1425 Evaluation of the Impact of Cigarette Smoke and THS 2.2 Aerosol Exposure on Tooth Coloration and Chemical Identification of Discoloration Markers

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Tobacco smoking is a risk factor for tooth discoloration. Pigmented compounds present in the particulate phase generated by combustion of tobacco may cause discoloration of dental hard tissues and restorative materials; the effect of exposure to aerosol from heated tobacco on tooth discoloration have not yet been explored. This study aimed to test if aerosol from a candidate modified risk tobacco product (MRTP), the Tobacco Heating System (THS) 2.2 (Philip Morris Products S.A.), had less impact on the color of dental hard tissues and composite resin restorations than cigarette smoke (CS) and, for the first time, to characterize potential markers of tobacco smoke-induced discoloration deposited on the enamel surface. First, 22 extracted human premolars restored with composite resins (Filtek™ Supreme Ultra) were exposed to cigarette smoke (3R4F reference cigarettes; University of Kentucky) and THS 2.2 aerosol for 3 weeks (56 min/day). The teeth were brushed with regular toothpaste every week, after which the enamel, dentin, and composite resins were measured for color. Second, for evaluation of discoloration markers, 60 bovine enamel blocks were exposed to the total particulate matter (TPM) of CS or THS 2.2 aerosol or to artificial saliva (control) for 2 weeks. Brushing (without toothpaste) and color measurements were performed every week. Noticeable discoloration of enamel, dentin, and composite resins was observed following exposure to CS (whole smoke or TPM). The discoloration was not distinguishable by eye ($\Delta E<3.3$) following exposure to THS 2.2 (whole aerosol or TPM), and no color mismatch between teeth and restorative materials was observed. To identify potential discoloration markers, surface-deposited chemicals were extracted using carbon disulfide solvent. Untargeted analyses using gas chromatography coupled to time-of-flight mass spectrometry were followed by PLS correlation against discoloration results obtained from CS TPM exposure (Pearson correlation of R^2=0.96). Eleven compounds were highlighted with a variable in projection (VIP) score above 2. Their identity searched through NIST17 library, after discriminant autocorrelation matrix of their mass spectral information, revealed a strong pattern association of the terpenoids family and no relationship to nicotine. In THS 2.2 TPM exposed samples, several of these compounds were also detected but at lower level, in line with the lower discoloration observed. Compared with CS TPM exposure, THS 2.2 TPM exposure resulted in lower deposition of color-related compounds on enamel surface, consistent with minimal discoloration of dental hard tissues and restorative materials.

1426 Computational Mining of Public Toxicogenomic Data Provides Insights into Liver Steatosis Adverse Outcome Pathway

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Exposure to certain chemicals can induce steatosis or fatty liver in humans. The adverse outcome pathway (AOP) framework aims to assist in the development of alternatives to animal testing, which will enable rapid screening and identification of such chemicals. In the current steatosis AOP, the activation of nuclear receptors, is considered the molecular initiating event (MIE) due to their ability to modulate lipid synthesis and transport. To be useful, however, this AOP needs to be evaluated using diverse steatogenic chemicals. Here we mined large, publicly available, toxicogenomic datasets obtained from rat *in vivo*, rat *in vitro*, and human *in vitro* studies involving exposure to 18 known steatogenic chemicals, including amiodarone and valproic acid, at different doses and time points of exposures. We identified differentially expressed genes (DEGs) associated with each chemical exposure, and then used them to perform pathway enrichment analysis and analyze the activation of steatosis MIEs. The most frequently identified DEGs in rat *in vivo* studies were those indicative of oxidative stress, such as *Aldh1a7*, *Aldh1a1*, *Akr7a3*, *Akr1b7*, and *Gsta3*, which are commonly associated with mitochondrial toxicity. Our MIE analysis revealed that exposure to these steatogenic chemicals led to the absence of activation of genes involved in lipid synthesis and transport, indicating that the causal links between the current MIEs and steatosis are insufficient. We identified eight DEGs (*Cyp1a1*, *Egr1*, *Ccnb1*, *Gdf15*, *Cdk1*, *Pdk4*, *Ccna2*, and *Ns5atp9*) and one pathway (retinol metabolism), which were conserved across the three test systems (rat *in vivo*, rat *in vitro*, and human *in vitro*). Such steatosis-relevant molecular mediators are likely to be relevant to human exposures. Collectively, our analyses suggest that mitochondrial toxicity is an important and overlooked MIE in chemical-induced steatosis. Our computational mining approach provides an important means to assess AOP development, identify knowledge gaps, suggest ways to improve AOPs, and aid the development of new screening tools.

1427 Linking Co-regulated Gene Modules with Polycyclic Aromatic Hydrocarbon-Related Cancer Risk (RPF) in the 3D Human Bronchial Epithelium

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Polycyclic aromatic hydrocarbon (PAH) exposures are linked to numerous adverse outcomes in humans, with cancer as the greatest concern. The cancer risk associated with PAH exposures is commonly evaluated using the relative potency factor (RPF) approach, which estimates PAH mixture carcinogenic potential based on the sum of relative potency estimates of individual PAHs, compared to benzo[a]pyrene (BAP), a reference carcinogen. This study set out to combine a novel 3D human *in vitro* bronchial epithelial cell (HBEC) model with transcriptomic and bioinformatic approaches to evaluate molecular mechanisms of direct relevance to PAH cancer risk. Significantly regulated genes from HBEC treated with PAHs and PAH mixtures were analyzed using a two-tiered WGCNA approach, first to identify co-modulated gene sets significantly correlated to RPF, and then to link genes to a more comprehensive list of regulatory values, including inhalation-specific risk values. Conditional Toxicity Value (CTV) Predictor was used to predict risk and toxicity values for chemicals present in mixtures with unavailable data. Over 3,000 genes from

cell cycle regulation, inflammation, DNA damage, and cell adhesion processes were found to be significantly correlated and co-modulated with increasing RPF (BAPeq). Benchmark dose modelling in BMDExpress 2 with a dose response of benzo[a]pyrene further identified DNA damage, cell adhesion, and cell cycle regulation (S phase) among the most sensitive pathways for gene regulation. Next, co-modulated genes were linked to additional cancer-relevant risk values, including inhalation unit risks and oral cancer slope factors. Gene modules significantly correlated in common between RPF, oral cancer slope factors, and IARC cancer categorizations were identified. These gene sets represent potential biomarkers that can be used to evaluate cancer risk associated with PAH mixtures. Here, we demonstrated a novel manner of integrating gene sets with chemical toxicity equivalence estimates through WGCNA to understand potential mechanisms. Similar studies could further inform cancer risk evaluations and assessments by incorporating organotypic human *in vitro* models with additional endpoints and risk values associated with chemical exposures.

1428 Utilizing Adverse Outcome Pathways as a Framework to Organize Evidence and Support Carcinogenicity Risk Assessment

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The ICH S1 guidelines relate to the assessment of human carcinogenicity risk for new pharmaceuticals. To satisfy this guidance, rodent carcinogenicity studies are routinely carried out. However, these tests are time consuming, expensive and use a lot of animals. Moreover, they do not always produce results of human relevance. In order to alleviate these shortcomings, recent proposals for changes to the guidance suggest using a weight of evidence (WOE) approach. This method involves collating evidence from other relevant sources, such as genotoxicity assays, chronic repeat dose studies and knowledge of pharmacology, in order to categorise human carcinogenic risk. The results may then negate the need for a rodent carcinogenicity study. One challenge in using WOE is the organisation and interpretation of this disparate information in a consistent way to reach a meaningful conclusion. A potential solution is to use an adverse outcome pathway (AOP) framework. This enables the organisation of knowledge and data in a transparent and logical manner to support decision making. In this work we utilised a previously developed network containing 38 AOPs for carcinogenicity and integrated assays, data, models and knowledge of human relevance in order to investigate the use of AOPs to support assessment. Overall, 70 assays were linked to appropriate key events. Several case studies were then examined, using assay data, *in silico* models and knowledge contained within the network to reach a conclusion and, where needed, reason between results when multiple pathways are implicated. The categorisation of these outcomes according to ICH S1 were then compared to results based on a conservative approach which uses unstructured data alone. Our work shows that evidence relating to carcinogenic risk can be organised into an AOP framework, making it easier to interpret and reason between data, models and knowledge. Preliminary validation shows that network analysis can allow for more accurate interpretation of carcinogenic risk than using unstructured data alone and thus reasonably negates the requirement for a rodent carcinogenicity study where appropriate. The approach also allows for additional knowledge beyond assay results to be captured, which may put conclusions into context and save time, money and animals.

1429 PhIP Exposure: Alzheimer's Disease Relevant Neurotoxicity

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by two hallmark pathologies: accumulation of extracellular senile plaques composed of amyloid-beta peptide (Aβ) and intracellular neurofibrillary tangles. AD is a major public health crisis given the prevalence and lack of curative treatment and thus, it is critical to identify modifiable risk factors that could decrease risk of disease or disease course. The role of the diet in AD has received recent attention, with several studies suggesting that high levels of meat consumption may increase AD risk. Much of our prior work has shown that exposure to heterocyclic aromatic amines (HAAs) produced Parkinson's disease (PD)-relevant neurotoxicity. Given mechanistic and pathological overlap between AD and PD, we investigated whether exposure to 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP), a prevalent dietary HAA formed during high temperature meat cooking, may produce AD-relevant neurotoxicity. In the present study C57BL/6 mice were treated with 100mg/kg or 200mg/kg PhIP for 8h (single oral gavage) or 75mg/kg for 4 weeks and 16 weeks (oral gavage, 3 times/week). PhIP exposure for 8h resulted in increased acetylcholine, oxidative stress, synaptic proteins and β-Site amyloid precursor protein cleaving enzyme 1 (BACE 1), in the hippocampus. PhIP exposure for 4 weeks resulted in increased BACE 1 and oxidative stress in hippocampus. PhIP exposure for 16 weeks resulted in increased BACE 1, Aβ aggregation, tau phosphorylation and oxidative stress in hippocampus, all hallmark features of AD. Quantification of intracellular nitrotyrosine revealed oxidative damage in cholinergic neurons after 8h and 16 weeks PhIP exposure. PhIP exposure did not induce inflammation in the hippocampus. Furthermore, SH-SY5Y cells and primary cortical neurons exposed to PhIP also exhibited increased BACE 1 and oxidative stress. Our study demonstrates that PhIP exposure induces neurotoxicity similar to that seen in AD. *In vitro* studies also imply that increase in oxidative stress and BACE 1 might be a possible mechanism by which PhIP promotes Aβ aggregation. Collectively, our study suggests a potential link between diets high in HAAs and AD.

1430 Exposure to an Environmentally Relevant Level of Copper in Drinking Water Alters the Microbiome in a Humanized APP Knock-In Mouse Model of Alzheimer's Disease

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While aging and genetics are the primary risk factors of Alzheimer's disease (AD), exposure to environmental factors are also known to modulate the disease pathology. Chronic exposure to copper is a putative environmental risk factor for AD. Recent epidemiological studies highlight that high serum levels of labile copper correlate with cortical thinning, accelerated cognitive decline, and earlier onset of AD. Although copper is an essential metal, environmental exposure to its inorganic cupric form (Cu^{2+}) may exert toxicity. However, exact toxic mechanisms of action of copper leading to AD remain largely undetermined. Here, we investigate its impact on the gut microbiome, as copper is a natural antimicrobial, and is normally ingested. Recent studies implicate gut dysbiosis in the onset and progression of AD in both humans and animal models. We hypothesized that chronic exposure to environmentally relevant copper through drinking water will alter a healthy composition of the gut microbiota in APP^{NL-G-F} knock-in (APP-KI) mice. Dysbiosis may perturb host metabolism and inflammatory homeostasis to contribute to AD neuropathology. Three-month-old male and female APP-KI and wildtype C57BL/6J mice were exposed to 1.3 ppm $CuCl_2$ in drinking water ad-libitum for 3 months. Fecal matter was sampled monthly and DNA was isolated for amplicon sequencing of the 16s rRNA V4 region. At sacrifice, blood and brain tissue were harvested. The brain was subject to standard techniques for immunohistochemistry and biochemistry. Plasma was analyzed for cytokines. Copper had no significant effect on the microbiota of WT mice. APP-KI mice had a higher Bacteroidetes to Firmicutes ratio due to an increase in the genus *S24-7* and decrease in the genus *Allobaculum* respectively. No significant differences were seen in plasma cytokines. Similarly, no significant change in amyloid plaque burden was detected in the brain of APP-KI mice. The microbiome of APP-KI mice is less resilient to effects of copper compared to WT mice. These results suggest that certain bacterial populations in the gut are sensitive to environmental agents and longer exposures with older animals may elucidate associations to AD.

1431 Chronic Exposure to Real-Time Traffic-Related Air Pollution Exacerbates and Accelerates Alzheimer's Disease Phenotypes in Wild-Type and TgF344-AD Rats

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Traffic-related air pollution (TRAP) has been linked to increased risk of Alzheimer's disease (AD) or dementia in multiple epidemiological studies. However, this association has yet to be confirmed preclinically, in part because reproducing chronic real-world TRAP exposures is challenging in animal models. To overcome this challenge, we exposed male and female TgF344-AD and wildtype F344 (WT) rats to real-time TRAP or filtered air (FA) from postnatal day 28 to 15 months of age in an exposure facility that drew air from an adjacent highway tunnel in northern California. This preserved the particulate, gaseous, and semivolatile components of TRAP, and exposed animals to human-relevant TRAP concentrations. We hypothesized that TRAP triggers neuroinflammation to accelerate or exacerbate AD-relevant outcomes. At 3, 6, 10, and 15 months of age, brain samples were collected and analyzed for amyloid plaque burden, neuronal cell loss, glial cell activation, and cytokine protein levels. Amyloid plaques were detected in the brain of TRAP-exposed

TgF344-AD female rats at 3 months of age, prior to plaque development in any group, and both male and female TRAP-exposed TgF344-AD rats showed increased plaque burden at 6 and 15 months of age compared to FA controls. Amyloid plaques were not detected in WT rats. ELISA revealed that TRAP also significantly increased levels of triton-soluble Aβ42:Aβ40 in TgF344-AD rats, a parameter that is highly correlated with cognitive decline in humans. Both WT and TgF344-AD rats also exhibited increased neuronal cell loss at 15 months compared to age- and sex-matched FA-exposed controls. Interestingly, male WT, but not TgF344-AD rats exposed to TRAP showed cognitive deficits at 15 months of age. TRAP increased IBA1, but not GFAP, immunoreactivity in rats of both sexes in both genotypes, suggesting selective effects on microglia vs. astrocytes. Levels of pro- and anti-inflammatory cytokines, as quantified by Luminex, were also altered in TRAP-exposed rats at 3 and 6 months of age in a sex-dependent manner. Collectively, these findings demonstrate that chronic TRAP exposure exacerbates and accelerates several AD-relevant endpoints, and suggest that these pathologic responses may be mediated by microglial activation. *Supported by the NIEHS (grants R21 ES025570, P30 ES023513 and T32 ES007059) and NIA (grant P30AG010129).*

1432 Distinct N6-Methyladenosine Profiles in mRNA Caused by $CoCl_2$ *In Vivo* and *In Vitro*

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N6-methyladenosine (m^6A), which mainly occurs in brain tissues and concentrates on mRNA of genes related to cortical neurogenesis, cell cycle and neuron differentiation, thus playing an important regulatory role in the development and function of the central nervous system. Our previous study found that expose to cobalt *in vitro* and *in vivo* would cause neurodegenerative damage. In this study, we used human brain neuroblastoma cells (H4 cell) ($CoCl_2$; 400μM) and animal models ($CoCl_2$; 16 mg/kg) to test the neurodegenerative changes induced by cobalt. We have carried out an unbiased global analysis of m^6A in mRNA of mouse temporal cortex and human brain neuroblastoma cells. We show that there are intriguing differences in these samples with respect to the degree of methylation, functional classification of methylated transcripts. Specifically, we observe a pronounced ccumulation of m^6A sites in the vicinity of the translational stop codon. We also analyze potential correlations between m^6A and RNA target sites, binding sites of RNA binding proteins. In summary, our data confirm that by altering the original m^6A level, $CoCl_2$ disrupts the expression of some key genes and affects some important signaling pathways, thereby participating in the neurotoxicity caused by cobalt chloride. Our study provides a new basis for the future research on the neurotoxicity caused by m^6A modification involved in cobalt chloride. *This work was supported by National Natural Science Foundation of China (No. 81573195; 81973083); Fujian Province Science and Technology Innovation Joint Fund Project (No. 2017Y9105).*

1433 Chronic Copper Exposure Perturbs Microglial Transcriptomics Signatures in Wild-Type and Mouse Model of Alzheimer's Disease

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Copper (Cu) is an essential metal that mediates a variety of biological reactions with its redox property. Cellular homeostasis of Cu is critical for human health as dyshomeostasis may lead to neuronal damage caused by subsequent impairment in biosynthesis of neurotransmitters, antioxidant defense, immune function, and synaptic transmission. Intriguingly, recent emerging evidence indicate that chronic exposure to Cu and its dyshomeostasis are linked to accelerated cognitive decline and increased risk for Alzheimer's disease (AD) and other dementias. We and others previously demonstrated that exposure to Cu through drinking water significantly increased parenchymal amyloid-beta (Aβ) plaques, aberrant microglial activation, and decreased endothelial low-density lipoprotein receptor-related protein 1 in mouse models of AD. In the present study, we continued to execute in-depth analysis of microglial transcriptomic changes and neuroinflammation in the brains of J20 transgenic mouse model of AD and wildtype (WT) littermate controls after exposing to 1.3 ppm Cu-containing drinking water for 3 to 5 months starting at 1 month of age. While the overall Aβ plaque burden at the early plaque stage of J20 mice remained unaffected by Cu exposure, microglial transcriptomes were markedly altered in Cu exposed mice even in pre-pathological stage at 4 months of age. The homeostatic signature genes were generally reduced while the cluster of degenerative genes was elevated in microglia-specific transcripts collected from intact brain tissues using translating ribosome affinity purification (TRAP) technique. Global RNA-seq analysis also revealed that inflammation- and cytokine-related genes were differentially expressed in microglia from Cu exposed mice. Notably, the Cu exposed mice also exhibited impaired cognitive function, which could potentially be mediated by exacerbated microglial dysfunction and neuroinflammation in the brain. These results collectively implicate that chronic Cu exposure triggered persistent transcriptomic and functional changes in microglia and induced neuroinflammation in either plaque-free WT mice or pre/early-plaque stage J20 mice.

1434 Discovery, Structure-Activity Relationship Study, and *In Vivo* Evaluation of Novel Series of Mono-amine Transporter Inhibitors

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Monoamines, such as dopamine (DA), serotonin (5-HT) and norepinephrine (NE), have an important modulatory role in neurotransmission in the central nervous system (CNS). They are involved in numerous physiological functions and pathological conditions. Therapeutic application of selective or mixed monoamine transporter (DAT, SERT or NET) inhibitors include a variety of neurologic disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, and others. The optimal selectivity profile varies between pathological conditions; however, the exact trend is not well understood yet. Thus, for direct comparison of produced effects, it can be beneficial to identify a class of compounds that includes structurally similar ligands with different selectivity profiles. Recently, we have serendipitously discovered a novel selective DAT inhibitor. Our compound UR118 showed strong DAT inhibition (Ki= 15 nM) with a moderate affinity to SERT (Ki= 1620nM). We design and synthesized a series of analogs of UR118 to initiate a structure-activity relationship (SAR) study. From the first series of analogs, we obtained selective DAT inhibitors (Ki= 40), as well as DAT-NET inhibitors (Ki= 6.5 and 167 nM, respectively). In addition to inhibition of transporters, UR118 blocked signaling of H1 (Ki= 32nM) and 5HT-2C (Ki= 335 nM) receptors. Further, this compound did not affect the remaining 45 GPCRs in the tested panel of CNS-related receptors. All tested analogs of UR118 have shown similar binding patterns with different selectivity profiles (data obtained from PDSP). Further, UR118 was found to moderately permeate the BBB (C_{max} = 1000nM, 10 mg/kg, i.p in mice) with the accumulation in adipose tissue, lung, and liver. UR118 was stable in rat liver microsome (63% remaining at 1 hr.). In pertussis-induced multiple sclerosis model (mice), we observed a significant improvement of symptoms in the UR118-treated group when compared to the vehicle-treated group of animals. In conclusion, we have discovered a novel series of monoamine transporter inhibitors with varying selectivity profile and favorable CNS-drug-like characteristics. Compound UR118 with DAT selectivity has shown definite therapeutic potential in *in vivo* model of multiple sclerosis.

1435 Interaction of Endoplasmic Reticulum Stress and Inflammation in Amyloid-Beta-Induced Neurotoxicity

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Amyloid-beta (Aβ) peptide accumulation in the brain is a pathological hallmark of Alzheimer's disease (AD). Amyloid-β protein has toxic effects on neurons and induces the activation of microglia as well as astrocytes. As evidences support the involvement of endoplasmic reticulum (ER) stress in AD and other neurological disorders; here we investigated the involvement of ER stress and its interaction with inflammation and glial cell activation in Aβ-induced neurotoxicity. $Aβ_{(1-42)}$ was administered bilaterally through intra-cerebro ventricular (icv) injection in the brain of Wistar rats. After completion of treatment, acetylcholinesterase (AChE) activity and amyloid aggregation was measured in hippocampus (HP) and cerebral cortex (CC) regions of rat brain. ER signaling proteins- glucose regulated protein 78 (GRP78), growth arrest and DNA damage-inducible gene 153 (GADD153) and eukaryotic translation initiation factor-2α (eIF2α), neuronal marker protein- microtubule associated protein-2 (MAP-2) and microglial marker protein- ionizing calcium-binding adaptor molecule-1 (Iba-1) were assessed by western blot. Pro-inflammatory cytokines viz. Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α) and Interferon-γ (IFN-γ) were measured using ELISA kits. Apoptotic death of neurons was determined by mRNA levels of caspase-12 and caspase-3. Histological changes were also observed. ER stress inhibitor salubrinal was used to assess the specific role of ER stress and its interaction with inflammation. $Aβ_{(1-42)}$ treatment caused a significant increase in AChE activity, amyloid aggregation, expression of ER stress marker proteins, microglial activation, cytokines levels, caspase-12 and caspase-3 mRNA levels which were attenuated with salubrinal treatment. $Aβ_{(1-42)}$-induced histological alterations and decrease in MAP-2 levels were also inhibited by salubrinal which indicates that ER stress interacts with other

pathological events leading to neurodegeneration. Results of present study implicate ER stress as potential molecular mechanism in Aβ-induced neuropathology which could further emerge as a possible therapeutic target in AD.

1436 Paraoxonase-2 Deficiency in the Aging Brain: Immunological and Neurodegenerative Consequences

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Paraoxonase-2 (PON2) is a member of the paraoxonase family consisting of PONs 1, 2 and 3. Unlike PON1 and 3 which circulate in the body associated with HDL, PON2 is a ubiquitously expressed intracellular antioxidant enzyme located at the inner mitochondrial membrane, where it maintains redox balance and manages oxidative stress generated during cellular respiration. PON2 deficiency has been shown to have adverse consequences in the CNS *in vitro*, with deficient astrocytes and neurons having higher levels of reactive oxygen species (ROS) and subsequent cell death. As oxidative stress is increasingly implicated in the etiology of neurodegenerative diseases, and PON2 deficiency is expected to cause higher oxidative burden based on *in vitro* studies, we sought to characterize PON2 deficiency *in vivo* in the aging brain and determine if lower PON2 levels predispose toward neurodegenerative disease. For this study, female and male wildtype (WT) and PON2 deficient (PON2 DEF) mice (N = 10 - 15 per group) were aged 18 - 24 months following a protocol approved by the University of Washington IACUC. After reaching aged status, brain regions (cerebellum, cortex, striatum, hippocampus) and other organs were collected for biochemical analysis. Multiple immunological and neurodegenerative targets at the transcript and protein level were compared between WT and PON2 DEF tissue, with significant sex-specific differences found in the cortex. PON2 DEF female mice had significantly lower mRNA levels of interferon gamma (IF-γ), tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 4 (IL-4) compared to WT, while PON2 DEF males only had significantly lower IF-γ and IL-1β. These results suggest a compensatory effect with PON2 deficiency, which may be reducing cytokine expression and subsequent inflammation in cortical tissue. Glucose transporter 4 (GLUT4) mRNA was also significantly higher in both female and male PON2 DEF mice, suggesting potential dysregulation of glucose transport pathways. Overall, the current findings suggest PON2 deficiency leads to significant changes in immunological and neurodegenerative related targets and supports further study of PON2 in the aging nervous system. *Supported by P42ES06496 and T32ES07032.*

1437 Exposure to Ultrafine Particulate Matter *In Utero* Impairs Memory Function in Adult Wild-Type and APP Knock-In Mice

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Reports indicate that exposure to the airborne particulate matter (PM) during development impairs cognitive function later in childhood. In mice, *in utero* PM exposure leads to notable neurodevelopmental changes in the embryo. However, limited work has been done to elucidate underlying mechanisms by which and to what extent *in utero* exposure to PM alters brain structure and function and accelerates cognitive decline. To determine to what extent and the critical mechanisms by which PM exposure during development mediates neurotoxicity and cognitive impairment in adulthood, here we exposed a humanized Amyloid Precursor Protein (APP) knock-in mouse model of AD and wildtype mice to concentrated ultra-fine PM *in utero*, allowing them to age, and examined the cognitive impact in adult mice. Cognitive outcomes are measured using the object location memory (OLM) and object recognition memory (ORM) tasks. Wildtype (wt) C57BL/6J female mice were crossed with wt or APP^{NL-G-F} knock-in males, resulting in either wt or APP^{NL-G-F} heterozygous pups. Following congenital plug observation, females were exposed to either concentrated quasi-ultra-fine particulates ($PM_{0.18}$) collected from the ambient air at UCI or filtered air by the versatile aerosol concentration enrichment system (VACES) at the UCI Air Pollution Health Effects Lab during gestation. Total exposure time was approximately 3 weeks, for 5 hours/day, 4 days/week. Pups were then allowed to age to 11 months. Mice were then tested in the ORM and OLM tasks. Mice were sacrificed for tissue harvesting the following behavior tasks, at 12 months of age. Brain tissues are processed using standard techniques for immunofluorescent section staining, protein probing, and RNA analysis. Wt mice whose mothers were exposed to $PM_{0.18}$ during gestation exhibited decreased performance in the OLM task compared to mice in the filtered air group. No differences were seen within the APP^{NL-G-F} group in OLM, or between genotypes. In the ORM task the $PM_{0.18}$ exposed APP^{NL-G-F} heterozygous mice performed worse than those exposed to filtered air group, but no differences were seen in the wildtype group. Behavior task results indicate that PM exposure *in utero* contributes to memory impairment in adult wildtype and AD model mice. Biochemical tests are ongoing to determine whether AD pathology or other molecular pathways leading to neurodegeneration are impacted.

1438 Lead and DDT Additively Increase the Levels of Amyloid Precursor Protein in Hippocampal HT22 Cells

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Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting over 5 million people in the United States. With the vast majority of AD cases being sporadic late onset of disease, there is rationale in investigating the potential role of the environment in the etiology of disease. We previously reported that serum levels of dichlorodipheyldichloroethylene (DDE), the metabolite of pesticide dichlorodiphenyltrichloroethane (DDT), were significantly higher in AD patients compared to age-matched controls. Likewise, it has been suggested that lead (Pb) exposure is a risk factor for AD based on multiple experimental studies in non-human primates and rodents. Both Pb and DDT have been reported separately to increase levels of amyloid precursor protein (APP) and increase amyloid-β (Aβ) in cells. Because these two contaminants were highly prevalent in the environment at the same time, we sought to determine whether they may interact to produce greater effects on the amyloid pathway. Here, we used the HT22 mouse hippocampal neuronal cell line, which has been shown to differentiate into cholinergic hippocampal neurons with N2 supplement. Exposure of HT22 cells to Pb, DDT or combinations of the two did not result in significant increases in APP levels, as determined by immunofluorescent staining. Upon differentiation of HT22 cells, we observed significant APP increases following 24 hr treatment with DDT and Pb separately and in combination; 1 μM DDT (63%), 10 μM Pb (98%), and 1 μM DDT and 10 μM Pb in combination (161%). Additionally, we found that after 48 hr of treatment, APP levels were further increased by 1 μM DDT (159%), 10 μM Pb (160%) and the combination (257%). These data demonstrate that Pb and DDT both increase APP levels and, that the combination of the two results in approximately an additive response. Taken together, these data suggest that Pb and DDT may act on similar mechanistic pathways affecting the amyloidogenic pathway. These data provide for a new understanding by which Pb and DDT may contribute mechanistically to AD risk. *Supported in part by NIH R01ES026057.*

1439 Modeling Gene-Environment Interactions in Amyotrophic Lateral Sclerosis (ALS): A Relevant Genetic Model and Low Manganese Exposure Levels Do Not Trigger Paralysis *In Vivo*

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset paralytic disorder with mostly unknown etiology but believed to result from gene x environment (GXE) interactions. Most previous ALS GXE studies used animal models with a full-blown ALS phenotype combined with environmental exposures that are already highly toxic alone. However, this scenario may not realistically model GXE interactions in ALS as patients do not report any signs of neurotoxicity before clinical onset of the disease. Here, to test the hypothesis that ALS results from the interaction between silent exposures and silent genetic susceptibilities, we used a knock-in mouse model of an incompletely penetrant ALS variant of TDP-43 "G298S". The heterozygous (het-G298S) knock-in mice are "ALS-silent" over their lifespan but the homozygous knock-ins show mild neuromuscular junction denervation at an advanced age (2.5 yrs.), confirming the validity of these models. Primary spinal cord cultures from wild-type (WT) and het-G298S mice were generated to screen for possible GXE interaction with various neurotoxic metals previously proposed to have a role in ALS. This pilot *in vitro* study suggested that G298S increased the vulnerability of motor neurons to manganese (Mn) exposure. Thus, WT and het-G298S mice were chronically exposed via drinking water to 200ppm Mn -a low level of Mn below those reported to be neurotoxic in past studies. Mice were exposed for six months starting in young adulthood (P145). To control for sex, this study included 50% females and 50% males and it was powered based on our past ALS mouse studies. Mice were tested weekly by treatment/genotype-blinded investigators for grip strength (loaded grid) and general locomotor activity (rotarod) over the six months of the study. At the end of the study, mice were tested with the CatWalk™ for gait and quantitative assessment of footfalls. Mild motor differences (CatWalk paw contact intensity, print length and area, swing speed [$p<0.025$, ANOVA] and lower loaded grid score [$p<0.05$, linear

mixed model]) were detected in het-G298S mice relative to WT but independent of Mn treatment. These results indicate that 200ppm Mn exposure is likely too low to initiate clinical ALS in our genetically vulnerable knock-in model -even though this level of exposure is at the high end for humans. While tissue pathology analysis is ongoing, a subsequent cohort is underway in which mice are exposed to 400ppm Mn to evaluate the effects of a higher exposure level to Mn.

1440 Role of Microglia in Diesel Exhaust Particulate Extract-Induced Neurotoxicity and Clearance of α-Synuclein Aggregates in the Larval Zebrafish Brain

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Parkinson's disease (PD) is the second most common neurodegenerative disease and is caused by a combination of genetic and environmental factors. The main hallmarks of PD include loss of dopaminergic neurons in the substantia nigra, aggregation of the protein alpha-synuclein leading to the formation of toxic Lewy bodies, and neuroinflammation. Epidemiological studies have reported a positive association between exposure to air pollutant (AP) and incidence of PD. We hypothesize that AP exposure activates microglia, interferes with alpha-synuclein processing and contributes to neuronal injury. Transgenic zebrafish (ZF) embryos were utilized to determine the effect of a major component of AP, diesel exhaust particulate extract (DEPe), on microglia's interactions with alpha-synuclein and their role in neurotoxicity. DEPe exposure led to a significant loss of dopaminergic neurons in the ZF brain and activation of microglia, seen morphologically and through preliminary rtPCR quantification of expression of inflammatory cytokine genes. Reduction of microglia using morpholino oligonucleotides did not alter DEPe-induced neuron loss. However, microglia were also activated with overexpression of GFP-tagged human alpha-synuclein in the ZF brain, and were found to engulf it, suggesting that they play a role in synuclein homeostasis and contribute to the neuroinflammatory response. ZF embryos treated with DEPe also had altered light-cycling behavior. These data suggest that microglia may not play a direct role in DEPe-induced neuron loss in our ZF model, but do play a role in clearing aggregated alpha-synuclein and contributing to neuroinflammation in PD. Current studies are focusing on the effects of DEPe-induced microglial activation on alpha-synuclein kinetics since both of these processes have been implicated to play a pathological role in PD. Additionally, we are utilizing a rat cortical cell culture model to confirm these findings, as well as begin to study the role that another key glial cell, astrocytes, play in DEPe's neurotoxicity. Ultimately, these studies will help elucidate the mechanisms by which AP contributes to neurodegeneration in PD.

1441 Multiplex Analysis in a *Drosophila* Gene-Environment Model Identifies Interactions among LRRK2, Rotenone, and α-Synuclein

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Epidemiological studies strongly support a role for environmental factors, particularly pesticide exposure, in the pathogenesis of Parkinson's disease (PD). Genetic risk factors, including penetrant single gene mutations and risk factors identified from genome-wide associated studies (GWAS) also contribute to PD risk and progression, but available model systems are limited in their ability to interrogate gene-environment crosstalk *in vivo* and at scale. Here we use *Drosophila* as a model system to understand complex gene-environment interactions in PD. We developed a multiplex model in *Drosophila* in which we knocked down GWAS candidate genes in neurons in a robust new α-synucleinopathy model and exposed the flies to environmental neurotoxicants. We identified an interaction between LRRK2, rotenone and α-synuclein. Expression of the disease-causing Lrrk-G2019S mutant in the presence of rotenone and alpha-synuclein induced behavioral deficits and mitochondrial dysfunction. Further, super resolution microscopy analysis revealed that the interaction of LRRK2, α-synuclein and rotenone leads to hyperstabilization of actin cytoskeleton. We have previously shown that LRRK2 has actin severing activity and previous studies have implicated the GTPase domain in regulating actin severing. We next expressed a GTPase domain mutant, Lrrk-Q1003H, designed to mimic the human protective mutant LRRK2-R1398H. Interestingly, expression of the LRRK2 protective mutant attenuated behavioral deficits mediated by LRRK2-rotenone-synuclein interactions. Further, expression of the protective mutant also attenuated the actin stabilization and mitochondrial deficits. Together, using our novel multiplex model in *Drosophila* we have identified an interaction between LRRK2, α-synuclein, and rotenone which is modulated by actin stabilization and mitochondrial dysfunction. Our findings have implication towards development of a personalized approach for drug discovery and lead identification.

1442 Mitochondrial Mechanisms of Harmane-Induced Dopaminergic Neurotoxicity in *C. elegans*

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Harmane (1-methyl-9H-pyrido[3,4-b]indole) is heterocyclic aromatic amine (HAA) that is a known tremorigen, and is also linked to Parkinson's disease (PD). It is found in cooked meat, roasted coffee beans, and tobacco. Our previous research showed that harmane produces selective dopaminergic neurotoxicity evidenced by decreased mitochondrial viability, elevated superoxide levels, and morphological alterations consistent with neurodegeneration. Given, that mitochondria are the primary target organelle, the goal of this study was to determine the mitochondrial mechanisms of neurotoxic action. Inhibition of 2-oxoglutarate carrier (OGC) ameliorated harmane-induced DA neurotoxicity. Specifically, OGC inhibition decreased harmane-induced effects on neuronal morphology and mitochondrial content. The effects appeared to be harmane specific since OGC inhibition failed to protect toxicity from other insults. Thus, we have identified OGC as critical to mediating harmane neurotoxicity. Further, N-acetyl cysteine, an antioxidant that also activates mitochondrial complexes and is a precursor for glutathione (GSH) ameliorated harmane neurotoxicity. Conversely, inhibition of glutamate cysteine ligase, a key enzyme in GSH biosynthesis increased neurodegeneration. Together, these data suggest that GSH levels are critical to harmane-induced DA neurotoxicity. In support of previously observed harmane-induced increases in superoxide, the mitochondrial targeted superoxide dismutase mimetic, XJB-5-131 ameliorated DA cell loss. Taken together, our findings suggest that OGC could be a possible entry port of harmane into mitochondria and that targeted scavenging of superoxide is beneficial. Additionally, our data also indicate the critical role of GSH to harmane neurotoxicity. HAAs are a widespread dietary exposure. Mounting data suggest HAA-induced neurotoxicity and our findings implicate specific mitochondrial mechanisms.

1443 Carnosine Reduces Toxic Effects of Manganese on Mitochondrial Membrane Potential

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Manganese (Mn) is an essential metal that at excessive brain levels causes manganism, which is similar to Parkinson's disease. The toxic mechanism of action is not fully understood. Proposed mechanisms suggest Mn causes oxidative stress and mitochondrial dysfunction causing damage to dopamine neurotransmission. Previously we showed Mn reduced mitochondrial O_2 consumption in gill of *Crassostrea virginica* and that two proposed treatments for manganism, EDTA and p-aminosalicylic acid (PAS), protected against this. We also showed Mn disrupts mitochondrial membrane potential of *C. virginica* gill lateral cells (GLC). We hypothesize the neurotoxic effects of Mn on GLC mitochondria membrane potential can be blocked by carnosine, an antioxidant with potential neuroprotective properties. To test this we used the mitochondrial selective fluorescent probe TMRM (tetramethylrhodamine methyl ester perchlorate) to study mitochondrial membrane potential in GLC. GLC were treated with 2.5μM TMRM, then exposed to either Mn (125 μM) alone, carnosine or PAS (125 μM) alone, Mn with carnosine (125 μM each), or Mn with PAS, and compared to control GLC. Mitochondrial fluorescence was viewed on a Leica fluorescence microscope with epilume illumination with a 50 watt HBO mercury excitation lamp and Texas Red filters (Ex 560 nm, Em 630 nm). Photomicrographs were taken with a Leica DFC400 camera at 100X and 200X at 0, 10 and 20 min. All sections were photographed with the same camera and microscope settings. GLC mitochondrial fluorescence intensity (FI) was measured with ImageJ from NIH. Control GLC fluoresced brightly indicating a strong mitochondrial membrane potential. GLC treated with carnosine or PAS alone had FI similar to controls. Mn treatment caused a 40% reduction in FI over the 20 min period, while GLC co-treated with Mn and PAS showed no loss. Carnosine was less effected than PAS in blocking Mn because carnosine co-treatments still allowed Mn to cause a 15% loss in FI compared to controls. This study showed PAS and carnosine have protective effects against Mn on GLC mitochondrial membrane potential, with carnosine being less protective than PAS. These findings should be of interest to those exploring possible therapeutic treatments for manganism. *This work was supported by grants NIGMS-2R25GM06003, NIH-K12GM093854-07A1 and PSC-CUNY 62344-00-50.*

1444 Carnosine Reduces Neurotoxic Effects of Manganese on an Oyster Gill Cilio-Inhibitory Dopamine System

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Manganese (Mn), a neurotoxin causing manganism a Parkinson's-like disease, disrupts dopamine (DA) neurotransmission. The neurotoxic mechanism is not fully resolved. Lack of effective treatment for manganism is a major obstacle for clinical management. Gill lateral cell (GLC) cilia of *Crassostrea virginica* are controlled by serotonin-DA innervations. DA is cilio-inhibitory; serotonin cilio-excitatory. Previously we showed acute and short-term Mn treatments block cilio-inhibitory effects of DA. This neurotoxic action of Mn is reversed by p-aminosalicylic acid and taurine. Recent reports suggest the dipeptide carnosine (*beta*-alanyl-L-histidine) is worth investigating as a neuroprotective agent for manganism because of its efficacy in other neurodegenerative diseases. We hypothesize carnosine will reduce neurotoxic actions of Mn on cilio-inhibitory effects of DA in *C. virginica* GLC. We conducted acute experiments testing DA on excised gill treated with Mn (10^{-4} M) in the presence or absence of carnosine (10^{-4}M). Cilia activity of GLC was measured by stroboscopic microscopy. Cilia of control GLC that were first activated by serotonin (10^{-5}M) responded normally to DA (10^{-6}-10^{-4}M) with the appropriate decrease in cilia beating from about 20 beats/sec to about zero. Mn disrupted the DA induced cilio-inhibitory dose response and carnosine treatments reduced this neurotoxic effect, generating a cilio-inhibitory response similar to DA without Mn. We also conducted short-term experiments in which *C. virginica* were treated with Mn (100 µM) for 2, 3 and 5 days with or without carnosine (100 µM). Control animals were sham treated. For each treatment period, co-treating animals with carnosine and Mn reduced the neurotoxic effect of Mn on the cilio-inhibitory DA system, generating cilia responses similar to sham treated controls. The study showed that in *C. virginica* carnosine reduced the neurotoxic effects of Mn on the cilio-inhibitory DA system. The findings are helpful in furthering the understanding of the neurotoxic mechanism of Mn and provides evidence suggesting carnosine should be further investigated as a potential therapeutic agent for manganism. *Supported by grants 2R25GM06003 of NIGMS, K12GM093854-07A1 of NIH and 62344-00-50 of PSC-CUNY.*

1445 G Protein-Coupled Inwardly Rectifying Potassium Channel (GIRK) and Control of Gill Lateral Cell Membrane Potential and Cilia Response of *Crassostrea virginica*

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Crassostrea virginica gill lateral cell (GLC) cilia are controlled by serotonin-dopamine innervations. Dopamine (DA) is cilio-inhibitory and hyperpolarizes the plasma membrane. Serotonin is cilio-excitatory and depolarizes. GLC DA receptors are D2-like (D2R). D2R activation opens G protein-coupled inwardly-rectifying potassium channels (GIRK) causing hyperpolarization. It is unknown if GLC have GIRK and if hyperpolarization is involved in cilio-inhibition of DA. We hypothesize GIRK is present in GLC and activation hyperpolarizes GLC and slows cilia beating. We conducted immunohistofluorescence (IHF) studies with GIRK antibodies and tested a GIRK inhibitor and activator on changes in plasma membrane potential while simultaneously measuring cilia beating using stroboscopic microscopy. Plasma membrane potential was measured using $DiBAC_4(3)$ and a microspectrum analyzer. IHF showed GIRK present in GLC. ML297 a GIRK activator hyperpolarized the membrane but had no effect on cilia beating. $BaCl_2$ a GIRK inhibitor had no effect on membrane potential or cilia beating. However applying DA after $BaCl_2$ decreased cilia beating but did not hyperpolarize the membrane indicating $BaCl_2$ was effectively blocking GIRK channels. In other experiments gills were treated with manganese (Mn), a neurotoxin causing manganism, a Parkinsons-like syndrome. In *C. virginica* Mn interferes with cilio-inhibition of DA, blocking both hyperpolarization and cilio-inhibitory in GLC. In the present study Mn had no effect on the GIRK activator or inhibitor, suggesting the toxic effect of Mn on the DA system is not involved in altering GIRK activity but rather activation of D2R. The study differentiates steps of D2R signaling pathway involved with cilia beating from those controlling plasma membrane potential. It also showed Mn does not affect GIRK activity in GLC. This is important because the mechanism of Mn neurotoxicity is not fully resolved and lack of effective treatment for manganism in humans is a major obstacle for clinical management. The findings are helpful in furthering understanding of D2R signaling pathway and Mn neurotoxicity, and provides evidence to guide future studies on therapeutic agents for manganism. *Supported by grants 2R25GM06003 of NIGMS, NIH K12GM093854-07A1 and PSC-CUNY 62344-00-50.*

1446 Effects of Manganese on Cilio-Inhibitory Actions of Dopamine D2 Agonists in Gill Lateral Cells of *Crassostrea virginica*

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Gill lateral cell (GLC) cilia of *Crassostrea virginica* are controlled by serotonin-dopamine innervations. Dopamine (DA) is cilio-inhibitory; serotonin cilio-excitatory. Manganese (Mn) a neurotoxin causing manganism a Parkinson's-like disease is characterized by aberrant DA neurotransmission. The mechanism of Mn neurotoxicity is not fully resolved. Lack of effective treatment for manganism is a major obstacle for clinical management. Previously our lab showed Mn disrupts cilio-inhibitory actions of DA in *C. virginica* and that post-synaptic DA receptors on GLC are D2-like (D2R). Since Mn interferes with D2R signal transduction pathway preventing normal cilio-inhibitory response, we hypothesized Mn would interfere with the pathway when D2R agonists are used. We tested dose responses of three different D2R agonists (piribedil, N-propyl piperidine and ergocryptine) on excised gill to determine their efficacy on reducing GLC cilia beating in the presence or absence of Mn. Results for each agonist also were compared to gill treated with or without Mn using the natural ligand DA. In the absence of Mn, each agonist was cilio-inhibitory, with N-propyl piperidine being the most effective, reducing beating rates similar to DA. Repeating the experiments with Mn (10^{-5}M) present caused a moderate reduction in cilio-inhibitory effectiveness of ergocryptine and piribedil, but did not cause a reduction in the cilio-inhibitory ability of N-propyl piperidine. The results support our hypothesis that Mn interferes with the cilio-inhibitory actions of D2R agonists, however not all three agonists were equally affected. Since cilio-inhibitory actions of two of the three agonists were impaired by Mn the results suggest Mn toxicity may not be targeting the DA molecule itself, but rather interfering with one or more steps of the D2R signal transduction pathway. In addition the three agonists had different cilio-inhibitory effectiveness and therefore likely to have different D2R binding capacities on GLC. This may explain their varied response to Mn, especially if Mn is directly interfering with ligand binding or activation of D2R. This study helps further the understanding of the neurotoxic mechanism of action of Mn and may be valuable in searching for therapeutic treatments for manganism. *Supported by grant NIGMS-2R25GM06003, NIH-K12GM093854-07A1 and PSCCUNY-62344-00-50.*

1447 LRRK2 Activation as a Common Feature of Parkinson's Disease-Associated Environmental Toxicants

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Environmental contaminants that cause mitochondrial dysfunction are associated with increased Parkinson's disease (PD) risk, such as pesticides rotenone and paraquat, and organic solvents trichlorethylene (TCE) and tetrachloroethylene (PERC). Despite the structural diversity of these environmental contaminants, they are each implicated as inducers of cellular oxidative stress. Recently, we reported that the protein kinase LRRK2, the most commonly inherited mutation in familial PD, exhibited aberrant kinase activity within dopaminergic neurons of the substantia nigra (SN), in rats exposed to rotenone or TCE. This toxicant-induced elevated LRRK2 kinase activity resulted in neuronal pathology that mirrored inherited *mutations* in LRRK2, such as vesicular trafficking dysfunction, endolysosomal impairment, and protein accumulation. Based on these data, we hypothesized that LRRK2 activation is induced by oxidative stress produced by environmental mitochondrial toxicants, which may influence cellular pathology, and contribute to the degeneration of dopaminergic neurons. We assessed this by comparing wild-type (WT), endogenous LRRK2 activation measured using a novel, validated proximity ligation assay (PLA) in a dopaminergic cell line (N27-A) following *in vitro* exposure to rotenone, paraquat, TCE or PERC. Each of these toxicants significantly induced LRRK2 kinase activity corresponding with elevated reactive oxygen species (ROS), measured by the fluorescent ROS indicator dye, dihydroethidium (DHE; $p < 0.001$). To assess whether LRRK2 mutations increase susceptibility to environmental toxicant exposure, we measured toxicant-induced ROS in CRISPR-edited 293 HEK cells that express the most commonly inherited LRRK2 mutation (G2019S). ROS production was elevated at baseline in LRRK2-G2019S HEK cells, and significantly exacerbated following application of either rotenone, paraquat, TCE, or PERC compared to WT ($p < 0.0001$). Interestingly, LRRK2 knockout HEK cells were robustly protected from toxicant-induced ROS production. Similarly, the small-molecule LRRK2 kinase inhibitor MLi-2 protected both dopaminergic and HEK cells from toxicant-induced oxidative stress. Collectively, these data indicate a role for LRRK2 interaction with common environmental contaminants that are associated with increased PD risk, and represents a possible point of susceptibility for individuals with inherited LRRK2 mutations.

1448 Manganese Exposure Stimulates the Release of Exosomes Containing Misfolded α-Synuclein by Impairing Endosomal Trafficking and Autophagic and Lysosomal Protein Degradative Machinery

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Environmental exposure to excessive manganese (Mn) increases the risk of chronic neurological diseases, including Parkinson's disease (PD). Aggregated α-synuclein (αSyn) is a key pathophysiological characteristic of PD. Oligomeric αSyn can be released from neurons by exosomes, facilitating the spread of misfolded proteins to neighboring cells, triggering a neurotoxic response. We discovered that Mn enhances the release of misfolded αSyn via exosomes from dopaminergic neurons, but the underlying molecular mechanisms are unclear. To understand the Mn-induced release of exosomal αSyn, we examined how Mn modulates endosomal protein trafficking and misfolded protein degradation. We exposed MN9D dopaminergic neuronal cells stably expressing human wild-type (WT) αSyn (MN9D-αSyn) to Mn (300 μM) for 24 h. Mn significantly suppressed expression of the key endosomal recycling protein Rab11a, both at the protein and mRNA levels, suggesting Mn downregulates endosomal recycling mechanisms, thus forcing late endosomes to mature into multivesicular bodies (MVBs). Moreover, ectopic expression of WT Rab11a significantly mitigated exosome release in untreated and Mn-exposed MN9D-αSyn cells, whereas ectopic expression of mutant Rab11a (S25N) increased exosome release. Intriguingly, our qRT-PCR, Western blot, and ICC analyses also revealed that Mn exposure upregulated mRNA and protein levels of Rab27a, a key endosomal protein that mediates exosome release through fusion of MVBs with the plasma membrane, suggesting Rab27a upregulation contributes to Mn-induced exosome release. Since aggregated αSyn get degraded via autophagic/lysosomal pathway, we examined if Mn impairs this pathway to promote exosomal αSyn release. Our Western blot analysis of from *in vitro* and *in vivo* studies shows Mn upregulated autophagosomal marker Beclin-1, but downregulated lysosomal marker LAMP2, suggesting impairment of autophagolysosome formation following Mn exposure. Results from other key lysosome assays, such as ICC, LysoTracker staining and Cathepsin D assay confirmed Mn-induced lysosomal dysfunction. Together, these novel findings demonstrate Mn compromises endosomal trafficking, autophagic/lysosomal impairment, thereby promoting exosomal release of misfolded αSyn. *NIH ES026892, NS088206, Eugene Linda Lloyd chair.*

1449 Drp1 Inhibition Attenuates Autophagy Impairment Induced by Alpha-Synuclein and Neurotoxicants

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Gene-environment interactions play an important role in the pathogenesis of Parkinson's disease (PD). Enhanced neurotoxicity has been documented when α-synuclein (α-syn) is combined with neurotoxicants. Impairment in autophagy-lysosomal function resulting in misfolded and aggregated α-syn protein may account for such neurotoxicity. We report here that blocking dynamin-related protein 1 (Drp1) improved autophagy flux in mammalian cell models of α-syn, paraquat (PQ) and manganese (Mn), leading to reduced protein aggregation when α-syn was combined with these toxicants. To block Drp1 function, we used three complementary approaches: siRNA-Drp1, overexpression of Drp1-dominant negative and the small molecule mitochondrial division inhibitor-1 (mdivi-1). By using autophagy reporter HeLa cells with stable expression of mRFP-GFP-LC3 treated with human α-syn preformed fibrils (PFF) and by quantifying immunoreactivity of LC3 and p62 in N27 neuronal cells with stable inducible expression of α-syn, we observed that Drp1 inhibition abolished autophagy impairment induced by α-syn. Consistent with its role in improving autophagy function, Drp1 inhibition reduced proteinase K-resistant α-syn aggregates, as well as exosome release and spread of α-syn pathology from neurons to neurons and from microglia to neurons. To gain additional mechanistic insights into this protection, we assessed mTOR activity by quantifying the levels of phosphor-4E-BP1, which is a downstream substrate of mTOR, in stable α-syn N27 cells. We observed that α-syn activated mTOR, and strikingly, knocking down Drp1 inhibited mTOR activity to an equivalent extent of rapamycin, an mTOR inhibitor. Through investigation of the effects of PQ and Mn on autophagy, we observed a striking blockade of autophagy flux by these toxicants at concentrations much lower than their LC_{50} values. We also confirmed that, in models in which α-syn was combined with either PQ or Mn, proteinase K-resistant α-syn aggregation was significantly reduced by Drp1 inhibition. In summary, our data support a common and prominent role of impaired autophagy in genetic and toxicant induced cell models relevant to parkinsonism. Furthermore, our observation that Drp1 inhibition improved autophagy flux in these models is novel and significant.

1450 Mitochondrial and Autophagolysosomal Dysfunction Induced by Heterocyclic Aromatic Amine Exposure Is Modulated by Neuromelanin Formation

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Neuromelanin is a pigmented product of dopamine catabolism present in human dopamine neurons in the substantia nigra. However, most preclinical PD models (i.e., rodents and *in vitro* cultures) lack neuromelanin. Thus, the role of neuromelanin in environmentally induced neurotoxicity is generally ignored. Our lab and others demonstrated a selective dopaminergic neurotoxic potential of a group of chemicals produced from high-temperature meat cooking, known as heterocyclic aromatic amines (HAA). Further, we recently showed that two HAAs, 1-methyl-9H-pyrido[3,4-b]indole (harmane) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), induce dose-dependent toxicity in human SH-SY5Y neuroblastoma cells; where neuromelanin formation (through ectopic tyrosinase expression) increased intracellular HAA levels and several metrics of HAA-induced toxicity. The primary goal of the present research was to elucidate pathogenic mechanisms by which neuromelanin formation modulates HAA-induced neurotoxicity. Post HAA exposure, SH-SY5Y cells (with and without neuromelanin formation) were evaluated for oxidative stress, autophagic flux, and mitochondrial function/morphology. Our data show that SH-SY5Y cells exposed to harmane and PhIP induced concentration-dependent oxidative stress, loss of mitochondrial membrane potential and lysosomal degeneration. The magnitude of each of these changes was significantly increased in neuromelanin forming cells. Additionally, using the Seahorse MitoStress assay, we demonstrated that neuromelanin-forming SH-SY5Y cells have heightened susceptibility to HAAs, with respect to mitochondrial function. For harmane, functional alterations were also associated with mitochondrial morphological changes. Lastly, we showed that harmane treatment disabled autophagic flux by increasing autophagy and reducing lysosomal activity, promoting dysfunctional mitophagy. Taken together, our data show a pivotal role of neuromelanin in modulating specific pathogenic pathways that underlie HAA-mediated neurotoxicity. Our data also suggest that, in general, dopaminergic neurotoxicity should be evaluated in the presence of neuromelanin to increase the translational potential of laboratory studies.

1451 The Role of Inflammatory Mouse lncRNA AK039862 in PQ-Induced Proliferation and Migration Inhibition through the Pafah1b1/Foxa1 Pathway in Co-culture Environments

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Emerging evidence has suggested a significant role of long non-coding RNAs (lncRNAs) in neurodegenerative diseases, including Parkinson's disease (PD). It has been identified that particular lncRNAs have a potential effect on PD progression induced by neurotoxins (such as paraquat) through damage and inflammatory responses to dopaminergic/microglia cells. However, the specific role and mechanisms of LncRNA in PQ-induced PD disease progression remain unclear. In the present study, we focus on the comprehensive effect of particular lncRNA in mixed and sophisticated brain environments. We found the interesting individual and interacted features of lncRNA AK039862 are involved in PQ-induced cellular functional effects through using two non-contact co-culture models to better simulate the coexistence of dopaminergic cells and microglia. First, we detected that AK039862 was involved in the neuronal injury process in PQ-treated mice and determined its regulatory mechanism *in vitro*. Interestingly, we found PQ significantly inhibits cell proliferation and migration *in vitro*. Further research indicates PQ-induced decreased expression of AK039862 can rescue microglia proliferation and migration via the AK039862/Pafah1b1/Foxa1 pathway. Meanwhile, AK039862/Pafah1b1/Foxa1 pathway also participated in the interaction between microglia and dopaminergic cells with PQ treatment in two non-contact co-culture models. In summary, our findings elucidated the role of AK039862 in PQ-induced neurodegeneration via Pafah1b1/Foxa1 pathway, and revealed the complex and diverse functions of inflammatory AK039862 in the communication between microglia and dopaminergic cells. *Project supported by the National Natural Science Foundation of China (Grant No. 81573195).*

1452 Paraquat Primes the Microglial NLRP3 Inflammasome via the Voltage-Gated Proton Channel Hv1

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Paraquat (PQ) is one of the most widely used herbicides and can increase the risk of developing Parkinson's disease (PD) by ~2.5 times. PQ treatment induces PD-like symptoms with selective nigrostriatal degeneration, aggregation of α-synuclein, and increased neuroinflammation in the rodent brain. PQ-mediated microglial activation leads to dopaminergic neurotoxicity, but its mechanism is not well-established. Hvcn1/Hv1 is a voltage-gated proton channel selectively expressed in microglia and other immune cells. It has been shown to be required for NADHP-oxidase (NOX)-dependent production of reactive oxygen species (ROS) under the pathological conditions. The purpose of this study was to determine the role of Hv1 in paraquat-induced neuroinflammation and neurodegeneration. Using a bioinformatic approach of published microarray data, we found that *HVCN1* mRNA levels were significantly elevated in the substantia nigra of post-mortem samples from PD patients compared to age-matched controls. Direct PQ treatment on the BV2 mouse microglial cell line and primary mouse microglia (PMG) induced *Hvcn1* mRNA levels by 2-3-fold. This was accompanied by significant increases in ROS levels and mRNA expression of the *Nlrp3* inflammasome. PMG isolated from global Hv1 knockout (KO) mice displayed significantly reduced production of ROS and mRNA levels of *Nlrp3* and *Il1b* following PQ treatment. We used western blotting to observe and quantify the protein levels of NLRP3, as well as the inflammasome-related proteins cleaved IL-1β and cleaved caspase-1. The levels of NLRP3 protein and cleavage of the IL-1β and caspase-1 protein were increased after PQ treatment of the PMG. These effects were abolished in the Hv1 KO PMG. Following a single injection of 10 mg/kg PQ to C57BL/6J mice, levels of *Hvcn1* mRNA and IL-1β protein were increased by 6-fold and 2-fold in the striatum, respectively. This effect was abolished in Hv1 KO mice. These data demonstrate that direct PQ treatment primes the microglial NLRP3 inflammasome through the voltage-gated proton channel Hv1.

1453 A Differential Gene Expression Study of Two Siblings with Genetic Risk for Parkinson's Disease

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Parkinson's disease (PD) is characterized by the loss of dopaminergic (DAergic) neurons in the substantia nigra. As a complex, multifaceted disorder, accumulating evidence substantiates the role of gene-environment interaction in PD etiology. We have previously reported a case of two siblings harboring identical compound heterozygous *PARK2* mutations that exhibited markedly different clinical phenotypes. The older brother displayed symptoms of parkinsonism by his mid-20's while the younger brother showed exercise-induced dystonia in his mid-30's with no further clinical progression through his late 40's. Neural progenitor cells (NPCs) derived from induced pluripotent stem cell (iPSC) lines generated from both siblings showed an increased sensitivity to copper and manganese. Hence, we set out to investigate the differential gene expression profile of iPSC-derived neurons from the two siblings that may account for the observed phenotypic difference. In the present study, we generated four independent iPSC clones from each sibling, differentiated them into floor plate DAergic lineages, and performed RNA-sequencing. The expression profiles were evaluated at days 11 and 25 of differentiation. Following comprehensive transcriptomic profiling, we chose 14 genes to validate by qRT-PCR from those differentially expressed between the brothers. Genes were selected based on those known to be associated with PD or changes in PD environmental risk, such as SLC18A2, GSTT1, and HLA-DRB5. Lineage-specific markers were also examined to ensure successful differentiation. We report that the qRT-PCR validation was in good agreement with results obtained from RNA-sequencing, with 11 and 12 of 14 genes validated in day 11 and 25, respectively. Of these PD associated genes, there were substantial differences in expression levels between the siblings' DAergic neurons ranging from 2-fold to even higher. Findings from this study will provide us with information to help explain the phenotypic discordance in the two siblings. Finally, further analyses will advance our understanding of how gene-environment interactions may increase an individual's susceptibility to or provide resistance from the onset of PD.

1454 Toward Understanding Intrinsic Characteristics of Dopaminergic Degeneration

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The selective loss of dopaminergic (DAergic) neurons is a hallmark of Parkinson's disease (PD) whether etiology is idiopathic, genetic or toxicant related. The mechanisms underlying this specific DAergic neurodegeneration remain elusive. 6-hydroxydopamine (6-OHDA) has been extensively used to model specific DAergic neurodegeneration. Levodopa, a precursor to dopamine (DA) that can pass through the blood-brain barrier, is clinically used to resolve some of the symptoms of PD by increasing DA production in remaining DAergic neurons. Several studies have reported the presence of 6-OHDA in both rat and human brains as well as in the urine of levodopa-treated PD patients. While suggested in the literature, the role of DA in potentiating the unique susceptibility of DAergic neurons is not thoroughly investigated or substantiated. We aim to test the hypothesis that DA is necessary and sufficient to render DAergic neurons susceptible to 6-OHDA. To pursue this, we took advantage of the genetically tractable *Caenorhabditis elegans* (*C. elegans*) worm model and expressed green fluorescent protein (GFP), tyrosine hydroxylase (*cat-2*) and/or the DA reuptake transporter (*dat-1*) into ADF serotonergic neurons while removing native tryptophan hydroxylase (*tph-1*) and the serotonin reuptake transporter (*mod-5*). This novel approach allows us to investigate the role of DA and its transporter on neurodegeneration in a completely different phenotypic cell background (neoDAergic neurons) in a live organism. Here, we show that the genetic knockout of DA production and the DA transporter in *C. elegans* protects against 6-OHDA-induced DAergic neurodegeneration. Increasing DA production by increasing cat-2 transcription in native DAergic neurons made these neurons more susceptible to 6-OHDA. We establish that wildtype ADF neurons showed no significant degeneration upon treatment with 6-OHDA. Interestingly, however, subjecting our neoDAergic ADF neuron expressing strain to 6-OHDA showed significant degeneration in these altered neurons. Investigating gene expression differences between native DAergic neurons and neoDAergic ADF neurons in the next steps of the study can further parse out the intrinsic characteristics of DAergic neuron susceptibility. Together our study shows DA is necessary and sufficient in conferring neuronal susceptibility to 6-OHDA.

1455 Acquired Dysregulation of Dopamine Homeostasis Reproduces Features of Parkinson's Disease

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Dopamine has the potential to act as an endogenous neurotoxin when its vesicular sequestration is impaired and it undergoes oxidation and enzymatic metabolism - two cytosolic processes that generate reactive oxygen species and reactive metabolites. Dysregulation of dopamine has been hypothesized to contribute to the enhanced vulnerability of nigrostriatal dopaminergic neurons to degeneration in Parkinson's disease. Here, we present findings from a novel *in vivo* rat model of acquired dysregulation of dopamine sequestration in nigrostriatal dopaminergic neurons through viral-mediated small-hairpin RNA interference targeting endogenous vesicular monoamine transporter 2 (VMAT2) expression. Utilizing an adeno-associated (serotype 2) virus (AAV2-shVMAT2), viral-mediated interference of VMAT2 expression resulted in a loss of VMAT2 protein expression in transduced dopaminergic cell bodies in the substantia nigra with a corresponding loss of VMAT2 protein in the striatal terminals, an increase in dopamine metabolism, and deficits in dopamine-mediated behaviors. This model results in nigrostriatal dopaminergic neurodegeneration that can be rescued through reintroduction of exogenous VMAT2, demonstrating that dysregulation in dopamine sequestration via loss of VMAT2 is sufficient to cause neurodegeneration. Analysis of pathogenic mechanisms of degeneration within viral-transduced dopaminergic neurons identified oxidative damage of macromolecules evidenced by a 26.29% increase in 4-hydroxynonenal (paired t-test, n=9, $p<0.05$) and a 27.36% increase in 3-nitrotyrosine (paired t-test, n=9, $p<0.05$). Proximity ligation assay was used to quantify a 28.35% increase in autophosphorylation of the Parkinson's disease-associated kinase LRRK2, which indicates increased LRRK2 activation (paired t-test, n=4, $p<0.05$). As an additional measure of LRRK2 activity, the amount of phosphorylated Rab10, a substrate of LRRK2, was quantified with a 23.52% increase in phosphorylated Rab10 (paired t-test, n=4, $p<0.05$). Although there was no significant increase in total α-synuclein, there was a 42.85% increase in phosphorylated α-synuclein (paired t-test, n=4, $p<0.05$) as well as a 90.9% increase in the interaction between α-synuclein and mitochondrial protein import protein TOM20 (paired t-test, n=3, $p<0.05$), which

indicates the formation of aberrant α-synuclein. This model demonstrates that a progressive acquired loss of VMAT2 expression in adulthood is sufficient to induce Parkinson's disease associated pathogenic mechanisms of degeneration and provides a novel model to test therapeutic interventions for Parkinson's disease.

1455a Effects of Formaldehyde Exposure on Human Neural Cell Using 3D Model *In Vitro*

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Alzheimer's disease (AD), as a chronic neurodegenerative disorder characterized by rapidly progressive cognitive dysfunction and behavior impairment, is the most common form of dementia which accounts for 60 to 70 percent of cases. Pathologically, AD is characterized by severe atrophy and neuronal loss, extracellular neuritic plaques composed mainly of aggregated amyloid-β (Aβ), and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein. Multilevel complex interactions between genetic, epigenetic, and environmental factors contribute to the occurrence and progression of AD. Formaldehyde (FA) is the simplest of the aldehyde which is a widespread environmental organic pollutant. Recent studies suggested that elevation of FA in the body by endogenous and/or exogenous exposure may play important roles in AD development. To investigate the role of FA exposure in AD development and progression in human cells *in vitro*, a three-dimensional (3D) human neural cell culture model for AD was used. This model was able to recapitulate key events of AD pathology including β-amyloid plaques and neurofibrillary tangles. Immortalized human neural progenitor cells (ReNcell VM (ReN)) were virally transfected with AD-related mutant genes including mutant APP and/or PSEN1 and enriched based on GFP and/or mCherry signals by Fluorescence-activated cell sorting (FACS). In order to mimic the restrictive environment of human brain and allow Aβ deposition and aggregation, the FACS-sorted ReN cells were differentiated into neurons and maintained in a 3D Matrigel culture system. The AD Model and primary ReN cells were treated with different concentrations of FA as well as vehicle control for 16 weeks. Expression of Aβ40 and Aβ42 were measured in 4, 6, 12 16 weeks by ELISA and cytotoxicity was evaluated by LDH (Lactate Dehydrogenase) activity assay. The results revealed that FA significantly elevates expression of Aβ40 and Aβ42 in a concentration-dependent manner both in AD model and ReN cells. It indicated that FA exposure may play a critical role in AD development and progression. Further study of the mechanisms involving in FA and Aβ, tau protein, histone modifications, chromatin assembly will be explored, which may provide more clues of AD etiology and new insight into AD potential therapeutic targets.

1456 Non-Dioxin-Like Polychlorinated Biphenyls Affect the Sensory System Leading to a Delayed Escape Response in Zebrafish Larvae

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Polychlorinated biphenyls (PCBs) are persistent legacy contaminants that bioconcentrate in fish and other predators. Fish are a major dietary source of exposure for humans. One of the most abundant PCBs found in aquatic biota and in human maternal plasma and amniotic fluid is the non-dioxin-like PCB153, yet its biological activity is not well understood. There is increasing evidence that PCB153 targets the nervous system and may be responsible for behavioral deficits following exposures in early development. In this study, we used the zebrafish larvae to investigate the effects of the non-dioxin-like congeners PCB153 and PCB138 on the neural circuitry during development, in comparison to the dioxin-like PCB126. We tested behavioral effects in PCB-treated fish by assessing the larval escape response. Our results show that in 6-day old larvae exposed to PCB153 or PCB138, the response latency times (M = 58.0 ms, SD= 30.7, and M = 57.0 ms, SD = 33.5, respectively), were dramatically greater than the latency in controls (M = 20.9 ms, SD = 20.0), $F(3, 747)$ = 125.4, $p<0.0001$. Notably, PCB126 does not alter the latency (M = 19.2 ms, SD = 20.1). The escape response involves a neuronal circuit with detection of the signal in auditory hair cells and other sensory neurons, and transmission to the Mauthner cells and reticulospinal neurons. By using transgenic zebrafish lines, immunohistochemistry, and electrical stimulation, which bypasses the sensory system and directly activates Mauthner neurons, we were able to show that the Mauthner cells are present (in all of the 50 samples inspected) and functionally transmit information downstream of the circuit ($t(87)$ = 1.045, p = 0.2989). Morphological analysis of hair cells and innervating neurons is currently underway. Non-dioxin-like PCBs may be disrupting synaptic transmission to the Mauthner cell by inducing swelling of afferent neurons and modulating neurotransmission. This study advances understanding of the mechanism of action of a ubiquitous environmental contaminant, providing mechanistic insight into a process affecting rapid escape from predators (ecological relevance) and proper neuronal development (human health relevance). *NIH 5P42ES007381 - The Boston University Superfund Research Program.*

1457 microRNA-124 Protects Neural Cells against Arsenic-Induced Endoplasmic Reticulum Stress and Cytotoxicity *In Vitro* and Is Associated with Neurodevelopmental Outcomes in Children

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Arsenic (As) exposure adversely affects neurodevelopment in children. Accumulation of misfolded proteins leads to endoplasmic reticulum (ER) stress response, if not relieved, cell death. Despite the potential role of ER stress for As-induced neurotoxicity, the underlying mechanisms remain poorly understood. This study aimed to identify the roles of microRNA(miR)-124, a novel ER stress suppressor, in the regulation of As-induced ER stress response and cytotoxicity in neural cells. We further aimed to link these *in vitro* findings to neurodevelopmental outcomes in children who were exposed to As. Using Quantitative RT-PCR and Cyquant assay, we showed that miR-124 protects against As-induced cytotoxicity in neural cells with concomitant suppression of As-induced ER stress. In addition, As-induced cytotoxicity was exacerbated in miR-124 knockouts generated by CRISPR-based gene editing compared to scramble control. Furthermore, we identified two miR-124 SNPs rs67543816 (p=0.0003) and rs35418153 (p=0.0004) that are significantly associated with a mental composite score calculated from the Bayley Scales of Infant Development III in Bangladesh children. Our study implicates As-induced ER stress as a crucial mechanism for the detrimental effects of As on neural cell function and neurodevelopment and identifies miR-124 as a potential preventative and therapeutic target against detrimental effects of As exposure in children.

1458 Effect of Placental-Derived Mesenchymal Stem Cells on Fetal Brain Developmental Defects Caused by Maternal LPS-Induced Intrauterine Inflammation

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Maternal bacterial infections during pregnancy represent a significant risk factor in preterm as well as neuropsychiatric disorders with a presumed neurodevelopmental deficit. One of the downstream syndrome, neuroinflammation, has been reported to be highly associated with numbers of neurological and pathological diseases, like schizophrenia, cerebral palsy, and autism. Our previous published data has been used lipopolysaccharide (LPS) as a model of clinical bacterial infection in pregnant animals like many other reports. We found that LPS induced counteracted pregnancy loss and alterations in thickness and lamination of the neocortex *in vivo*. It is due to LPS-induced inflammation response, which is proved by application of N-acetyl-cysteine (NAC). However, the NAC is debatably used in clinical in pregnant subjects. Accordingly, mesenchymal stem cells (MSCs) are considered as a substitute therapeutic agent. MSCs are pluripotent cells that are present in multiple tissues, including bone marrow, adipose tissue, skin, muscle, blood, and placenta. It has been proved that MSCs can interact with cells of both the innate and adaptive immune systems, and thus act as a possible clinical therapy for immune-mediated diseases, such as osteogenesis imperfecta, severe graftversus-host disease (GvHD), and Crohn's disease. Therefore, it is conceivable that MSCs could protect the fetal brain against LPS caused inflammatory response. MSCs were directly infused into fetal abdomen or placenta at a density of 1×10^5 cells at 15 days of gestation age, and one hour later a dose of 25 μg/kg LPS was injected into pregnant rats intraperitoneally. To label the new generating neurons, 5-bromodeoxyuridine solution (BrdU, 50 mg/kg/day in PBS) was injected subcutaneously from 16 to 18 days of gestation age. Twenty-four hours later, the pregnant rats were sacrificed, and maternal hematology, cytokine and ROS production were analyzed. H&E staining and immuno-labeling of cortical laminate markers, such as Ctip2, Satb2, were used to investigate the fetal brain morphology and laminal development. Indeed, we found that LPS-induced toxicity could be ameliorated in the presence of MSCs, including in recovery of hemoglobin level and WBC number, an increase of cortical thickness, and prevention of abnormal Satb2 cortical lamination. In the first place, this study describes the initial efforts to evaluate the likely therapeutic effects of MSCs on LPS-induced developmental failure.

1459 Neonatal Exposure to Organophosphorus Flame Retardant TDCPP Induces Hippocampal Injury via Microglia-Mediated Inflammation

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Tris(1,3-dichloro-2-propyl) phosphate (TDCPP) is a phosphorus-based flame retardant common in consumer goods and baby products. Neurodevelopmental toxicity is a major concern of OPFRs' adverse health effects. However, the mechanism and early response for TDCPP-induced neurotoxicity are largely unknown. This study investigates the role of microglia-mediated neuroinflammation in TDCPP-induced neurotoxicity in mouse hippocampus and primary culture cells. C57BL/6 pups were administered with TDCPP (0, 5, or 50 mg/kg/day) via oral gavage from postnatal days 10 to 38 (28 days). The results showed that TDCPP exposure for 28 days altered the gene expression of neuronal markers *Tubb3, Nefh, Nes* and *Mbp*, and led to apoptosis in the hippocampus. The mRNA levels of pro-inflammatory factors *Il-1β, Tnfa* and *Ccl2* dose-dependently increased in the hippocampus at both 24 hours and 28 days following exposure, accompanied by microglia activation characterized by an amoeboid-like phenotype. In *in vitro* studies using the primary microglia isolated from neonatal mice, exposure to TDCPP (0-100 μM) for 24 hours resulted in cellular activation. It also increased the expression of genes responsible for inflammatory responses including surface markers and pro-inflammatory cytokines in a dose-dependent fashion. Neurite outgrowth of primary mouse hippocampal neurons was inhibited by treatment with the conditioned medium harvested from microglia exposed to TDCPP. These results reveal that neonatal exposure to TDCPP induces neuronal damage in hippocampus through microglia-mediated inflammation. The study suggests that microglia is a sensitive responder for OPFRs exposure.

1460 *D*-Amphetamine, but Not Desipramine, Impairs Sustained Attention and Short-Term Remembering in Mice Exposed to Low but Not High Doses of Methylmercury during Adolescence

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Methylmercury (MeHg), a known environmental neurotoxicant, has been previously shown to alter behaviors related to development of dopamine systems, such as observed impairments in choice and behavioral flexibility. Other behaviors related to development of dopamine systems, such as attention and memory, which are components of attention-deficit/hyperactivity disorder, may also be targets of MeHg's toxicity. Because adolescence is the final period for dopamine development prior to adulthood, changes in sustained attention and memory as a result of adolescent MeHg exposure were observed here using a mouse model. Mice were exposed to 0, 0.3, or 3ppm MeHg throughout adolescence and trained in a visual signal detection task in adulthood. Behavior was probed with dopaminergic and noradrenergic agonists, *d*-amphetamine and desipramine, in order to determine the specificity of MeHg's effects. MeHg did not alter baseline responding on the visual signal detection task but behavior for the 0 and 0.3ppm exposure animals was disrupted by the highest dose of *d*-amphetamine, 1.0mg/kg. Animals exposed to 3ppm MeHg were not impaired by any dose of *d*-amphetamine. Further, desipramine did not impair behavior for any animals in this task supporting hypotheses that performance in this task is specifically mediated by dopamine activity. Because animals exposed to 3ppm MeHg were not affected by *d*-amphetamine, this study suggests that interactions between MeHg and *d*-amphetamine are nonmonotonic.

1461 Zebrafish Larval Locomotor Activity Is Depressed by Lack of Swim Bladder inflation or Dimethyl Sulfoxide

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The US Environmental Protection Agency (US EPA) is evaluating alternative methods to screen and prioritize chemicals for developmental neurotoxicity. Assessing larval zebrafish photomotor response is recognized as a higher throughput testing strategy to identify neurotoxic chemicals. Usually, dead and malformed embryos are not included in the analyses of the larval zebrafish behavior, but, often, swim bladder inflation status is not assessed or reported. In our studies, we have determined that the photomotor responses of control larvae without inflated swim bladders is reduced >50% during the light phase of testing as compared to locomotor activity of control larvae with inflated swim bladders. Another variable, dimethyl sulfoxide (DMSO), a commonly used vehicle for administering chemicals, has been recently reported (Teixidó *et al*. 2018) to affect swim bladder inflation and locomotor activity in larval zebrafish, however larvae without inflated swim bladders appeared to have been included in that behavioral analysis. The main objective of the present study was to determine whether those behavioral changes noted by Teixidó and coworkers were a result of impaired swim bladder inflation or solely due to DMSO exposure. Embryonic zebrafish were developmentally exposed to a range (0.05-1.5%, v/v) of DMSO and, at 6 days post-fertilization, swim bladder inflation and locomotor activity were assessed. Larvae developmentally exposed to the highest DMSO concentration (1.5%) showed decreased incidence of swim bladder inflation, whereas locomotor activity for larvae with inflated swim bladders was altered at DMSO concentrations as low as 1%, a concentration used in some developmental neurotoxicity assays. These results indicate that DMSO can affect both swim bladder inflation and locomotor activity, but the effect on locomotor activity is the more sensitive endpoint. Therefore, caution should be taken when interpreting reports that include larvae without inflated swim bladders or utilize DMSO concentrations near 1% as both of these variables can influence behavior results. *This abstract may not necessarily reflect official Agency policy.*

1462 Lead Exposure Alters the Neurogenic Potential of Pluripotent Stem Cells

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Lead (Pb) is a teratogen that poses health risks after acute and chronic exposure. Developing fetuses are particularly sensitive to maternal blood lead levels because lead can freely pass through the placental membrane. Although lead's effect on late-stage embryonic development has been studied extensively, only a few studies have examined how lead affects stem cell determination and differentiation. In our present study, we sought to investigate how lead affects the retinoic-acid induced determination and differentiation of pluripotent stem cells into neurons. To achieve this end, we cultured P19 stem cells with 1-3μM Pb^{2+} and measured lead's effect on neurosphere formation, cytotoxicity, cell proliferation, and stem cell differentiation into neurons and glial cells. Moreover, we explored the mechanism of this affect by measuring N-cadherin and Sox2 expression after lead treatment. We discovered that lead exposure significantly decreased both the number and size of neurospheres by as much as 50% through an increase in cytotoxicity which decreased the number of alive cells by 40% after 48hrs when compared to the control. Moreover, lead exposure decreased expression of N-cadherin during the determination process by over 50% and significantly increased the number of Sox2-positive cells when lead was added during the determination process by over 60% when compared to the control. We also found that the negative effect of lead exposure on neuronal differentiation of stem cells did not significantly differ when we exposed the cells to lead during the process of determination or differentiation. However, lead exposure affected glial cell differentiation in a stage-specific manner by inhibiting glial cell differentiation when lead was added during determination and promoting glial cell differentiation when lead was added during the differentiation process. In summary, lead exposure during the development process may alter the differentiation potential and survival of neuronal stem cells and likely affects the overall development of the nervous system in developing embryos.

1463 2,3,7,8 Tetrachlorodibenzo-[p]-Dioxin-Induced Activation of the Aryl Hydrocarbon Receptor Alters Brain Morphology and Disrupts Oligodendrocyte Precursor Development

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor with roles in the development and function of the nervous system. Environmental contaminants such as dioxin (TCDD; 2,3,7,8 tetrachlorodibenzo-[p]-dioxin), polycyclic aromatic hydrocarbons (PAH), and polychlorobiphenyls (PCB) activate AHR and produce transcriptional changes that disrupt central nervous system development (CNS) and alter behavior. Our goal is to determine how AHR activation in zebrafish leads to hyperactivity by assessing changes in brain development and function. Developmental toxicology in studies in mice have demonstrated that a single prenatal exposure to TCDD disrupts CNS myelination by oligodendrocytes. Myelin insulates axons and is necessary for effective communication between neurons and impairment is associated with neurodegenerative disorders such as cerebral palsy and

multiple sclerosis. In the zebrafish hindbrain, the transcription factor Sox9b directs development of oligodendrocyte precursor cells. Our preliminary data indicate that toxicant-induced AHR activation (1 ppb TCDD; waterborne at 4 hours post-fertilization (hpf) for 1 hour) results in the downregulation of *sox9b* in the developing zebrafish brain. We hypothesize that AHR agonist exposure disrupts oligodendrocyte development due to impaired expression *sox9b*. Using fluorescent immunohistochemistry and confocal microscopy, we found that early exposure to TCDD altered brain morphology and resulted in a change in oligodendrocyte precursor number. To elucidate the roles of sox9b in early oligodendrocyte development, we have generated a dominant negative Sox9b (dnSox9b) to manipulate Sox9b function in developing zebrafish employing the Gal4/UAS system. Our preliminary results indicate that inhibiting Sox9b function significantly alters brain morphology and reduces oligodendrocyte number. Together, these studies demonstrate the effects of AHR activation on oligodendrocyte development and facilitate our understanding of how environmental contaminant exposure impact neurodevelopment.

PS 1464 Population Variability in Neurotoxicity Outcomes Modeled *In Vitro* with Diversity Outbred Neural Progenitor Cells

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Developmental neurotoxicity (DNT) is critical, yet challenging, health effect of chemicals to study. To date, there are few screens for DNT and existing assays do not incorporate sufficient genetic diversity to capture variation in DNT susceptibility across individuals in a diverse human population. To overcome these challenges, Diversity Outbred (DO) mice were created as a population resource composed of genetically distinct individuals, mimicking the diversity of the human population. DO mice allow investigators to generate toxicodynamic variability factors (TDVFs) that can replace default uncertainty adjustment factors for risk assessment. Here, we utilized 200 neural progenitor cells (NPCs) derived from DO mice to assess the population-wide variability upon exposure to six chemicals with DNT potential (0-100 µM): rotenone, dieldrin, estradiol, methyl mercury, 2,2′,4,4′,5-pentabromodiphenyl ether or isopropylated phenyl phosphate. Cell viability was measured at 114 h post-exposure. We observed wide distributions of the cytotoxicity EC10 for rotenone and methyl mercury, indicating that genetic variants contributed to interindividual sensitivities to these agents. Chemical-specific TDVFs were calculated to quantitatively assess population variability in toxic response using Bayesian statistics. The TDVFs for rotenone and methyl mercury were greater than default uncertainty factor, indicating that the reference dose for these chemicals may not adequately protect genetically sensitive subpopulations. In addition, mouse-derived TDVFs were comparable to human cell-derived TDVFs, suggesting that DO-derived TDVFs adequately capture human variability. TempO-Seq transcriptomic analysis, along with machine learning, is ongoing to determine mechanisms underlying and biomarkers associated with susceptibility to methyl mercury toxicity. Taken together, this population-based *in vitro* assay using DO NPCs provides a data-driven estimate for interindividual toxicodynamic variability suitable to inform risk assessment and more precise determination of human reference doses.

PS 1465 Embryonic and Larval Zebrafish (*Danio rerio*) Developmental Toxicity following Zinc Exposure

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The widespread application of emerging nanomaterials has made it necessary to question the potential adverse effects of nanoparticles released into the environment. The size and unique properties of nanoparticles have proven beneficial as ingredients in consumer products, but the literature has linked exposure to certain nanoparticles with adverse health effects ranging from oxidative stress to developmental toxicity and genotoxicity in aquatic environments. Zinc is an essential trace element and plays an important role in aquatic physiological processes, but in excess can display toxic effects. Bioaccumulation of zinc oxide nanoparticles (ZnO_2NP) has been observed in the gills of adult zebrafish, resulting in increased presence of reactive oxygen species (ROS). The toxicity of zinc derivatives ($ZnCl_2$ and ZnO_2, ZnO_2NP) has been attributed to the dissolution of the zinc ions as opposed to a nanoparticle-mediated toxicity. This study measures developmental toxicity in embryo and larval zebrafish (up to 18 dpf) with $ZnCl_2$ and ZnO_2NP at 0ppm, 0.25ppm, 0.50ppm, 0.75ppm, 1.00ppm, and 1.25ppm dosing concentrations in order to elucidate the potential effects of nanoparticle toxicity. Endpoints being measured include escape and avoidance behaviors, length, spinal angles, and ocular distance. Biochemical assays are also applied to measure mitochondrial activation and RTqPCR for genomic endpoints. Higher levels of avoidance behavior were observed following ZnO_2NP exposure when compared to $ZnCl_2$, while length, spinal angles, and ocular distance results were similar for both toxicants. Biochemical assays revealed increased mitochondrial activity with the nanoparticle when compared to chloride. RTqPCR revealed changes in SOD1, Bcl-2, Pink1 and p53. Bcl-2 and Pink1, genes related to mitophagy, suggesting the role of the mitochondria in zinc toxicity. In order to further confirm the toxicant role of the nanoparticle in zinc toxicity, further studies should include investigation of zero valent zinc nanoparticles along with dissociation of ZnO_2NP and $ZnCl_2$ into Zn (II).

PS 1466 The Use of Genetically Encoded Calcium Indicators to Understand the Effects of Neurotoxic Compounds on Neuronal Function

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Zebrafish are an outstanding model for studying the molecular mechanisms underlying brain development. Researchers have leveraged the throughput capabilities of the model to screen the neurotoxic potential of a large number of compounds. These powerful screens provide a readout of dysfunction, but do not identify the regional changes in brain activity that mediate the observed behavioral changes. Here, we describe how genetically encoded calcium indicators can be used to perform functional neuroimaging in zebrafish and how we can use functional neuroimaging to gain insight into how neurotoxicant exposure affects brain health. To visualize changes in regional brain activity following toxicant exposure, we are using the CaMPARI transgenic zebrafish line. CaMPARI, which stands for **Ca**lcium **M**odulated **P**hoto**a**ctivatable **R**atiometric **I**ntegrator, is a photoconvertible protein that permanently converts cells with high calcium from green to red in the presence of 405 nm light. In the transgenic line we are using, CaMPARI is driven by a differentiated neuron-specific promoter. Since neuronal firing is accompanied by an influx of calcium, following photoconversion the more active cells will appear red, while less active cells will remain green. To photoconvert CaMPARI in freely behaving fish, we modified our Noldus DanioVision behavioral unit to include a 405 LED. As an initial proof-of-concept experiment, we acutely exposed zebrafish larvae at 4 days post fertilization (dpf) to either (1) the GABA-inhibitor pentylenetetrazol (PTZ), (2) the anesthetizing agent MS-222, or (3) untreated embryo rearing medium. As expected, PTZ exposure increased motor activity and calcium signaling, whereas the acute exposure to MS-222 decreased motor behavior and neuronal activity. After successfully generating an experimental pipeline for photoconversion and analysis, we exposed larvae to varying concentrations of perfluorooctanesulfonic acid (PFOS), a pervasive toxicant that has been shown to produce hyperactivity in low doses in larval zebrafish and has also been shown to alter juvenile zebrafish behavior. Chronic exposure to PFOS (32 µM and 16 µM; from 4 hpf until the time of photoconversion) resulted in a significant global increase in neural activity and either hyper- or hypoactivity depending on the PFOS dose. We are currently examining the effects of different per- and polyfluoroalkyl substances as well as known neurotoxicants on neuronal activity. Moving forward we will collaborate with computational biologists to analyze regional differences in activity within individuals to identify the systems levels changes that are produced by toxicant exposure. Together, our functional neuroimaging studies paired with behavioral analysis will provide insight into the effects of neurotoxicants on brain function and provide a means for determining if different regional patterns of brain activity can produce similar behavioral responses.

PS 1467 Mutations Link Polycyclic Aromatic Hydrocarbon Exposure to Neurodevelopmental Diseases

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Polycyclic aromatic hydrocarbons (PAHs) are a mutagenic class of chemicals produced from burning organic matter. Inhalation and ingestion are the most common routes of exposure to PAHs, which are ubiquitous in air pollution, cigarette smoke, and burnt food. While epidemiologic studies show a clear association between PAHs and neurodevelopmental diseases, the underlying mechanisms are poorly understood. One possibility is environmentally induc*ed mutations*, which are well known to underlie cancer, but are seldom studied in the context of neurodevelopmental disease. Once metabolically activated by phase I enzymes, PAHs form mutation-inducing DNA adducts. To characterize mutational signatures relevant to cancer, Kucab and colleagues (2019, PMID 30982602) performed whole genome sequencing (WGS) of PAH dosed human induced pluripotent stem cells. We used this WGS dataset to explore the ability of PAHs to mutate neurodevelopmental genes. To determine if the genes mutated by PAHs were enriched for nervous system processes,

we performed gene ontology analysis of PAH-induced coding sequence variants. To determine if PAH-caused *mutations* promote specific nervous system diseases, we compared the number of PAH mutations in high-confidence gene sets for several diseases with 1,000 bootstrapped control gene sets from the genome. Out of 13 enriched gene ontology terms, 8 were closely related to neurodevelopment. Compared to control gene sets, there were 3.6-fold more mutations in autism genes, 4.0-fold more mutations in schizophrenia genes, and 3.0-fold more mutations in attention deficit hyperactivity disorder (ADHD) genes. Alzheimer's and amyotrophic lateral sclerosis (ALS) genes were mutated at similar rates to control genes. When the analysis was restricted to protein coding sequence mutations, autism and schizophrenia genes were mutated significantly more than randomly sampled genes, while ADHD, Alzheimer's, and ALS genes were not. Disparities in the mutability of genes may depend on gene length, gene expression, chromatin structure, and other genomic properties. Ultimately, we show increased susceptibility of neurodevelopmental genes to PAH induced *mutations*, which underscores the role of the environment in neurodevelopmental disease. Environmental exposures may be responsible for a large proportion of *de novo* mutations underlying sporadic cases of autism and schizophrenia.

1468 Identifying Key Events That Drive Neurotoxicity in Larval Zebrafish with Transcriptomic Dose-Response Modeling

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Gene expression data from short-term *in vivo* studies shows efficacy in quantifying concentration-dependent effects that inform toxicity outcomes, particularly for chemicals with limited toxicity data such as Per- and Polyfluoroalkyl Substances (PFAS). Here, zebrafish were exposed to 4.4-44.8 µM potassium perfluorohexane-1-sulfonate (PFHxS), 0.28-5.0 µM perfluorooctane sulfonic acid (PFOS), 0.25-5.0 µM Heptachlor (control for developmental neurotoxicity; DNT) or 0.4% DMSO daily from 0-5 days post fertilization (dpf). Automated behavioral assessments on 6 dpf revealed hyperactivity for all compounds at non-teratogenic concentrations. To identify key events that drive DNT, zebrafish were exposed to 7.87-25.1 µM PFHxS, 0.88-2.8 µM PFOS, 0.49-1.57 µM Heptachlor, or 0.4% DMSO. RNA was isolated from head tissue collected at 4 and 5 dpf, before the onset of hyperactivity, for sequencing (NextSeq 500). Differentially expressed genes were identified using Gene Specific Analysis (Partek Flow) and mapped to human orthologs for analyses (IPA). PPAR was predicted as a major regulator for all chemicals. Enrichment in neurologic-related pathways, including axonal guidance signaling and dopamine feedback, was also identified. Dose estimates from transcriptomic benchmark dose modeling (BMD_T, BMDExpress 2.0) identified median BMD_T values of 18 µM (PFHxS), 2 µM (PFOS), and 1 µM (Hetachlor) at 4 dpf and 10 µM (PFHxS), 1 µM (PFOS), and 1 µM (Heptachlor) at 5 dpf. Median BMD_T values were comparable to *in vivo* lowest observed effect concentration (LOEC) values for hyperactivity: 14 µM PFHxS, 0.88 µM PFOS, or 0.88 µM Heptachlor. This approach demonstrates that transcriptomic points of departure can be linked to hyperactivity (i.e. a functional DNT toxicity outcome) in larval zebrafish to ultimately inform mode of action delineation and enhance chemical risk assessments. *This abstract does not necessarily reflect US EPA policy.*

1469 Development of Automated Neurotoxicity Assay in Juvenile Mice

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The immature brain in the developing period is known to be vulnerable to environmental chemicals. However, reliable and efficient behavioral testing methods to directly assess the toxicological effects on cognitive function in juvenile animal models are limited. Here, we aimed to develop an automated and standardized method for assaying cognitive behavior of juvenile mice, as early as weaning, up to adult mice. Methods: We utilized IntelliCage (TSE Systems/New Behavior), an automated behavioral testing system, by customizing its hardware to suit the size of juvenile mice at different stages. A total of 24 juvenile mice (12 males and 12 females) were prepared using 15 pregnant mice of C57Bl/6N strain. To minimize the possible litter effects, one or two offspring of the same sex were obtained from each dam. On postnatal day 21, the mice were weaned, implanted with a microchip, and group-housed in the IntelliCage. The cognitive behavior of the mice was continuously assessed using a self-paced behavioral flexibility test (SP-FLEX) protocol until the mice became 8 weeks old. This test required mice to acquire place learning and shuttling rule and adapt to serial reversals, to obtain water reward effectively. The present method using a customized IntelliCage was found to detect juvenile mouse behaviors (corner visit, nose poke, and licking) without complications. All 3-week-old mice fulfilled the criteria of acquisition of the original place learning and shuttling rule (30% of correct response rate) within the first 48 h, and completed at least 15 reversal phases within the first 2 weeks. In addition, sexually-dimorphic behavioral choice tendencies in the SP-FLEX was found to emerge during the first 2 weeks. All the above results including the sexual difference found in juvenile mice are similar to those typically observed in adults over 8 weeks old. We successfully developed an automated cognitive assay for juvenile mice up to adult mice. This method can be applied to examine the safety of chemical agents in the juvenile period and to investigate the detailed time course of typical/atypical cognitive-behavioral development in mice. *This study was supported by JSPS KAKENHI JP18H03036.*

1470 Using Zebrafish to Study Birth Defects: The Effect of Valproic Acid during Embryonic Neurogenesis

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Valproic acid (VPA) is an anticonvulsant drug used to treat certain types of epilepsy. Several reports have shown that VPA has teratogenic effects during pregnancy. Fetal exposure increases the risk for birth defects, including neural tube defects (NTDs) and other congenital malformations, as well as learning difficulties and behavioral problems. We investigated neurotoxic effects of VPA exposure using zebrafish as a model organism. Different transgenic (Tg) fish embryos were exposed to increasing concentrations of VPA from 6 hours post fertilization. Tg (ngn1: eGFP) was used to visualize neural tube development in zebrafish embryos. Tg (mnx1: GFP) was used to study motor neurons and neurite sprouting. Tg (nestin: eGFP) was used to study neuroepithelial stem cells and neural progenitor cells. Our results showed that VPA exposure resulted in NTDs, motor neuron abnormalities and neurite sprouting defects. Additionally, VPA decreased the fluorescence of neuronal progenitor cells in early developmental stages, indicating fewer cells. We further investigated whether Vitamin B9 (Folic Acid, FA) could rescue the VPA-induced perturbations. Our preliminary results indicated that FA treatment did not rescue the number of the neuronal progenitor cells. We conclude that VPA exposure induces specific neurotoxic effects in developing embryos, and that zebrafish is a promising model to study these effects and to screen for countermeasures.

1471 Evaluation of Chemical and Nonchemical Interactions on Neurological Development of Rat Offspring

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Exposure to chemicals and non-chemical factors that occur during susceptible stages of neurodevelopment have been associated with several adverse outcomes in children, including cognitive deficits, anxiety, and depression. However, it is unclear as to how exposure to both chemical and non-chemical factors may impact brain development. To better understand these potential interactions, we exposed pregnant Long-Evans rats to Manganese (Mn), a reported neurodevelopmental toxicant, through drinking water (0, 2, or 4 mg/ml) from gestational day (GD) 7 to postnatal day (PND) 22. Half of the animals were perinatally exposed to a variable stress paradigm from GD13 to PND9. Measurements of somatic development and behavior were then quantified in the offspring to evaluate the impacts of Mn alone, and when combined with maternal stress. Mn concentrations in the drinking water provided to the dams were measured as < 0.001, 1.95 and 3.93 mg/ml respectively. Our results indicate no evidence of maternal toxicity was observed, and all dams gained weight at the same rate. Maternal stress and/or Mn exposure did not affect litter size but PND22 pup weight was significantly reduced in the 4 mg/ml Mn-exposed group. Water consumption was decreased in the Mn exposed dams in a dose-dependent manner but was not altered by the stress paradigm. The efficacy of the manipulations to increase maternal stress levels was determined using serum corticosterone (Cort) as a biomarker. A baseline concentration was established prior to treatment (GD7) and then measured at subsequent time points (GD16, PND9). Baseline Cort levels were low and similar in all treatment groups. As expected, Cort levels were elevated in all the Stress groups on GD16 when compared to the Non-Stress groups. A preliminary analysis of Mn tissue concentrations revealed levels elevated similarly in the brain and blood of Mn-exposed pups at PND2. Similarly, PND23 Mn-exposed dams also showed increased Mn concentrations in the brain, blood and liver; the addition of stress did not significantly alter the Mn levels in these tissues.

Taken together, these results show that maternal stress did not exacerbate the effects of Mn exposure on these measurements. *This abstract does not necessarily reflect US EPA policy.*

1472 Assessing Adult Cognitive and Motor Function in Three Genotypes of Mice Exposed to Benzo[a]pyrene during Early Brain Development

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Benzo[a]pyrene (BaP) is a carcinogenic polycyclic aromatic hydrocarbon commonly found in traffic-related air pollution, tobacco smoke, and grilled foods. BaP is linked to learning deficits and to neurodevelopmental delays in human and animal studies. We are using a mouse model to determine if genetic differences increase susceptibility to BaP exposure during early brain development. Mice lacking the CYP1A2 metabolic enzyme and wild type control mice were exposed to BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). A battery of cognitive and motor function tests were performed when the mice reached young adulthood (P60). We used a pole climb test to assess motor function, open field locomotor to assess activity levels, and novel object recognition to assess non-spatial visual learning and memory. We found a significant main effect of genotype in the earlier intervals with higher spontaneous activity in *Ahr^{d}Cyp1a2(-/-)* mice compared with the other two lines ($P < 0.05$). There was a significant gene x treatment interaction with the time to turn ($P < 0.05$) with BaP-exposed *Ahr^{b}Cyp1a2(+/+)* wild type mice having longer latencies whereas BaP-exposed *Ahr^{d}Cyp1a2(-/-)* mice had shorter latencies than corn oil-treated controls ($P < 0.05$). There were no significant differences in the time to descend the pole. There was a significant gene x treatment interaction with *Ahr^{d}Cyp1a2(-/-)* exposed mice showing impairments in object recognition memory ($P < 0.01$).

1473 Developmental Exposure to Non-Aroclor PCB 11 Affects Maternal Gestational Weight Gain, Male Offspring Birth Weight, and Hippocampal Apoptosis in Neonatal Mice

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Commercial production of polychlorinated biphenyls (PCBs) has been banned for decades and environmental levels of congeners present in Aroclor mixtures have been declining. However, recent studies have identified the presence in environmental and biological samples of other PCB congeners that were not present in the original commercial mixtures. PCB 11 (3,3'-dichlorobiphenyl) is one such congener that has been identified in air, water, dairy products, seafood, and meat. Elevated levels of PCB 11 have been detected in the serum of women at increased risk of having a child with a neurodevelopmental disorder (NDD). Previous *in vitro* studies using primary rat hippocampal and cortical neuron-glia co-cultures have shown PCB 11 enhances dendritic arborization and axonal growth at concentrations as low as 1 fM without affecting cell viability. Similar changes in these late stages of neuronal maturation have been noted in ASD patients. However, whether PCB 11 also influences earlier stages of neurodevelopment, such as neuronal apoptosis, which are also implicated in ASD pathogenesis has not yet been tested. There is also a paucity of data as to whether the developmental neurotoxicity of PCB 11 observed *in vitro* is recapitulated *in vivo*. Given that the diet is a major route of PCB exposure, we tested whether dietary exposure to environmentally relevant levels of PCB 11 during gestation and lactation in mice would affect hippocampal apoptosis in the neonatal period. Wild-type female C57BL/6J mice ($N = 5$/group) were given PCB 11 solubilized in peanut oil and mixed into peanut butter at doses of 0.1 or 1.0 mg/kg$_{BW}$/day beginning 14 days (d) prior to mating and continuing through gestation (19-20 d) and lactation (21 d). A vehicle control (VEH) group was given peanut butter with peanut oil alone. Unexpectedly, we observed that 1.0 mg/kg PCB 11 significantly increased maternal weight during both pre-conception and gestational dosing periods. Birth weight, postnatal weight gain, and anogenital distance (AGD) of male offspring in the 1.0 mg/kg group were also significantly increased, but not in any other group. At postnatal day (PND) 4, 1 male and 1 female pup from each litter were euthanized to quantify apoptosis. There was a significant increase in the total number of apoptotic cells in the hippocampus labeled via TUNEL staining in the 0.1 mg/kg group of both sexes. Taken together, these data indicate PCB 11 disrupts neurodevelopment in neonatal mice by increasing apoptosis and suggest a potential role of PCB 11 in endocrine disruption, evidenced by alteration of both maternal and male offspring weight and AGD. *Supported by NIEHS (R01 ES014901-09S1A1).*

1474 Maternal Exposure to Organophosphate Flame Retardants Alters Locomotor and Anxiety-Like Behavior in Male and Female Adult Offspring

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Endocrine disrupting compounds (EDC) are compounds found in our environment that interrupt typical endocrine function. A particular group of EDCs are flame-retardants due to their interaction with steroid and nuclear receptors in *in vitro* investigations. Humans are consistently exposed to flame-retardants daily as they are used in everyday items such as plastics, clothing, toys, and electronics. In the past, polybrominated diphenyl ethers have been used, however, since 2004, they have been replaced with organophosphate flame-retardants (OPFR) as the major flame-retardant chemical. The effects of maternal or developmental exposure to OPFR on behavior are currently underexplored. Yet, previous research in rodent models utilizing a commercial flame-retardant mixture containing OPFR reported alterations in anxiety like behavior in the elevated plus maze (EPM). Here we asses anxiety-like behavior in the open field test (OFT), the Light/Dark box (LDB), as well as, the EPM in adult offspring that were maternally exposed to OPFR or oil controls. Outcomes from the OFT and LDB indicate that males and females maternally-exposed to OPFR exhibit altered locomotor activity. Results of the EPM were sex-specific as we did not observe an effect in females; however, effects in males differed depending on treatment condition. Males maternally-exposed to OPFR exhibited an anxiolytic-like phenotype in contrast to their vehicle counterparts. This effect in OPFR-treated males was not due to alterations in locomotor activity. Our research illustrates that there are sex- and treatment-dependent effects of OPFR exposure on locomotor and anxiety-like behaviors in a mouse model.

1475 Manganese Consumption and Moderate Perinatal Stress: Complex, Persistent, and Sex-Dependent Changes in Neurobehavior as Assessed by Acoustic Startle Reflex, Prepulse Inhibition, and Choice Reaction Time in Rats

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The developmental effects of chemicals that co-occur in vulnerable populations with elevated physical and psychological stress is of increasing concern to risk assessors. In order to assess causality of these factors we developed a rodent model of co-occurring perinatal manipulations and conducted a series of assessments to evaluate behavioral and cognitive functions. Manganese (Mn), a potential neurodevelopmental toxicant, was delivered in drinking water (0, 2, or 4 mg/ml Mn) to pregnant, Long Evans rats from gestational day (GD) 7 to postnatal day (PND) 22. A variable paradigm designed to physically or psychologically challenge the dams was applied to half of the animals from GD13 to PND9. Here we report behavioral assessments performed in adult offspring. The acoustic startle reflex (ASR) and habituation differed between males and females, but no effects of stress or Mn were observed. Assessments of prepulse inhibition (PPI) of the ASR yielded a significant interaction between Stress, Mn, and prepulse intensity. Additional analyses suggested the 2 Mn group differed between stress groups at the 3 dB prepulse. Preliminary data from cued and uncued choice reaction time (CRT) assessments revealed female rats in the stressed group had longer decision times (0 and 4 Mn) and movement times (0 and 2 Mn), while reaction times in males was not affected. Choice accuracy in female rats was not affected by Mn but uncued accuracy was reduced by stress. Male accuracy on the cued task was reduced by Mn but not stress. Finally, premature responding was not affected by any treatment in either sex. This data supports a relationship between persistent perturbations of behavioral processes following early transient environmental perinatal stress and Mn exposure. The behavioral changes of these measures are complex, sex specific, and dependent on the specific task. *This abstract does not necessarily reflect US EPA policy.*

1476 One Size Does Not Fit All in Thyroid Disruption: Distinct Thyroid Hormone Profiles and Adverse Outcomes after Developmental Exposure to PTU (Propylthiouracil), DE-71 (Brominated Flame Retardant), and OMC (Octyl Methoxycinnamate)

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Correct thyroid hormone economy is crucial for mammalian brain development. In humans, even mild, non-clinical hypothyroxinemia during pregnancy has been linked to reduced IQ and increased risk of neurobehavioral disorders in the offspring. Several groups of environmental chemicals can interfere with thyroid hormone economy, which raises concern about their potential to disrupt human brain development. Here, we tested how developmental exposure to three thyroid-disrupting chemicals with differing mechanisms of action could affect thyroid hormone economy and cause adverse outcomes. We exposed 104 pregnant Sprague Dawley rats from gestational day (GD) 7 to postnatal day (PD) 22 to either corn oil (vehicle control), the anti-thyroid drug PTU (1 or 2.5 mg/kg/day), the UV-filter OMC (375 or 500 mg/kg/day) or a commercial mixture of brominated flame retardants DE-71 (20 or 40 mg/kg/day). Serum hormones (T3, T4 and TSH), body-, and organ weights were measured in dams and offspring at different times during the study. Our results showed that the three different compounds showed a distinct pattern of effects on liver, thyroid and brain, which seemed closely related to their primary mechanism of action. PTU exposure increased thyroid gland weight, DE-71 caused liver weight increases whereas reductions in brain weights were seen in OMC exposed offspring. The three compounds also caused very different effects on thyroid hormone economy and the hormone effect patterns varied in dams and offspring over the time course of the study. Thus, in developmental toxicity studies it is important to assess thyroid hormone economy at several time points throughout the study, in order to obtain better understand the chemically induced changes and to best protect humans from the adverse effects of thyroid hormone disruption. Future studies should focus on elucidating the origin of the different effect patterns and link them to possible adverse effects on the developing brain.

1477 Triclosan Impairs Mitochondrial Form and Function in the *Xenopus laevis* Tadpole Nervous System

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Mitochondrial health is critical for normal brain development, and in a toxic environment mitochondria reduce energy production, undergo mitophagy, and exclude themselves from their vast network. Recent studies have shown that triclosan, a widely used antibacterial and antifungal agent, acts as a mitochondrial uncoupler, which causes damage to the mitochondrial membrane, inhibiting energy production. The impact of triclosan on mitochondrial function in whole animals has not been thoroughly investigated, however. We performed a series of *in vivo* experiments designed to assess the impact of triclosan on mitochondrial form and function in *Xenopus laevis* tadpoles, with emphasis on the nervous system. We injected tadpole brains with TMRM, a fluorescent reporter of mitochondrial membrane potential. After two hours, we imaged the brain using high resolution confocal microscopy to obtain a baseline level of TMRM fluorescence, then immediately treated tadpoles with either triclosan (concentrations from 50μM to 0.1μM), the well-characterized uncoupler FCCP (0.5μM), or control, and then continued imaging the brain once every five min for 30 min. We found that 50μM triclosan significantly decreased TMRM fluorescence in mitochondria in the end feet of radial glial cells in ~ 15-20 min; lower concentrations operated over a longer time course. Preliminary experiments with injections of MitoTracker show that mitochondrial networking is substantially disrupted 20 min post onset of treatment with triclosan, similar to treatment with FCCP. Ongoing experiments with globally isolated tadpole mitochondria using Seahorse are assessing triclosan's impact on overall energy production by measuring oxygen consumption rates. In addition, the same batch of isolated mitochondria are being used in experiments using blue native-PAGE to assess sub-mitochondrial structures called supercomplexes. These aggregates consist of the major components of the electron transport chain and contribute to respiration efficiency. In toxic environments supercomplexes become disassociated, adversely affecting mitochondrial function. In summary, our results demonstrate that triclosan can act as a mitochondrial uncoupler in neural tissue of live animals.

1478 Folic Acid and Methionine Levels Affect Neural Differentiation and Compound Toxic Potency in the Neural Embryonic Stem Cell Test (ESTn)

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One of the challenges in the transition to animal-free testing is making sensible *in vitro* to *in vivo* extrapolations. However, culture conditions may affect the sensitivity of the *in vitro* test. In this study the influence of two important nutrients in culture medium that are part of one-carbon metabolism, folic acid (FA) and methionine (Met), was examined in the neural embryonic stem cell test (ESTn). ESTn mimics neural differentiation, from gastrulation until neural maturation. Both FA and Met are important for proper closure of the neural tube and affect differentiation of ESTn. Both FA and Met showed a dose-dependent effect on differentiation as assessed by morphological scoring. Low concentrations of Met enhanced cytotoxicity while ESTn deprived of FA showed only reduced differentiation without affecting cell viability. Interestingly, the concentrations of FA or Met for optimal neural differentiation were higher than normally present in the culture medium. Next, ESTn with slightly lowered FA (from 9 to 5.25 μM) or Met (from 20.1 to 16.31 μM) medium levels were exposed to methotrexate (MTX), a known developmental neurotoxicant that interferes with the one-carbon cycle. Lower FA and Met concentrations were chosen in such a way that ESTn differentiated with 90% efficiency relative to the control medium. Exposure to MTX at ID_{50} as determined in ESTn in control medium (39 nM) led to severe disruption of morphology in presence of low FA or Met, showing that ESTn became more sensitive to MTX toxicity. Exposure to a lower MTX concentration (13 nM) did not affect morphology but did increase protein expression of pre-neural tube marker E-cadherin and neural crest cell marker Twist2 in lower Met, but not in lower FA or control medium. In conclusion, both nutrients are important for proper differentiation and sensitivity of ESTn, and Met seems to be more essential in this regard than FA. In addition, it is suggested that the concentration of FA and Met in the medium may not be the optimal concentrations for ESTn. This study illustrates that basic culture conditions affect the read-out of *in vitro* tests, and therefore the outcome of *in vitro* to *in vivo* extrapolations.

1479 Evaluating Neonatal Reflexes in Mice Exposed to Benzo[a]pyrene during Early Brain Development

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Polycyclic aromatic hydrocarbons (PAHs) are a major component traffic-related air pollution, cigarette smoke and produced during baking and grilling food. They are a risk factor for learning and memory deficits in children exposed *in-utero* or during early life. We used the prototypical PAH benzo[a]pyrene to determine if there is genetic susceptibility to PAH-induced developmental neurotoxicity. Using a mouse model with variations in the aryl hydrocarbon receptor and CYP1A2, we compared monoamine neurotransmitter levels in offspring of dams treated with 10mg/kg/day benzo[a]pyrene. Negative geotaxis and righting reflex are innate behavioral responses in mice that require normal vestibular sensory abilities and neuromuscular control. In this study, we compared three genotypes of mice, *Ahr^{d}Cyp1a21(-/-)*, *Ahr^{b}Cyp1a2(-/-)* and wild type *Ahr^{b}Cyp1a2(+/+)* mice. Mice were treated with 10mg/kg/day BaP or the corn oil vehicle from gestational day 10 to weaning at postnatal day 10. The surface righting reflex was measured at P5, P7 and P10. Negative geotaxis was assessed at P7, P10 and P14. For the righting reflex, there was a trend for a significant gene x treatment interaction on postnatal day 5 (P5) with BaP-exposed pups from both *Cyp1a2(-/-)* lines have longer latencies ($P = 0.1$). There was a significant main effect of genotype on postnatal day 7 (P7) with both *Cyp1a2(-/-)* lines having shorter latencies than wild type mice. There were no significant differences on P10. For negative geotaxis, there were no significant differences at postnatal day 7 (P7). There was a significant main effect of treatment on P10 with BaP-exposed pups having shorter latencies than pups from corn oil-treated dams ($P < 0.01$). There was a significant main effect of genotype on P14 with *Ahr^{b}Cyp1a2(-/-)* knockout pups having longer latencies compared with the other two genotypes ($P < 0.05$).

1480 Effects of Developmental Exposure to Manganese and Nonchemical Environmental Factors on Learning and Memory, Social Interaction, Motor Activity, and Anhedonia

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Manganese (Mn), a toxicant that naturally occurs in the environment, has been shown to produce neurotoxic effects on the developing young when levels exceed physiological requirements. To evaluate the effects of this chemical in combination with non-chemical factors pregnant Long-Evans rats were treated with 0, 2, or 4 mg/ml Mn in their drinking water from gestational day (GD) 7 to postnatal day (PND) 22. Half of the dams received a variable stress protocol from GD13 to PND9. (Beasley et al. abstract). One male and 1 female offspring from each litter was tested on a selection of behavioral tasks beginning at PND17. Motor activity was measured in figure-eight mazes at PND 17, 29, and 79 (different group of animals at each age). Learning and memory was examined on PND34 using a Novel Object Recognition (NOR) task; social interaction was tested at PND 48 in a separate set of offspring. Anhedonia, a measure of depression, was assessed at PND53 by comparing the preference for chocolate milk versus water. In the NOR task, for males, the 4 mg/ml Mn no stress group showed a lower percentage of visits to the novel object compared to the 0 and 2 mg/ml Mn no stress groups. In females, the 2 mg/ml Mn stress and 4 mg/ml Mn no stress groups spent a greater percentage of time at the novel object than the 0 mg/ml Mn no stress group. The 4 mg/ml Mn no stress group also spent more time at the novel object than the 4 mg/ml Mn stress group. Preliminary observations show no changes on motor activity, social interaction, or anhedonia tests. There were statistically significant interactions between Mn and stress but the effects differ by sex and endpoint. These data indicate that other factors, extraneous but coexistent with chemical exposures, should be considered in the evaluation of chemical risk. *This abstract does not necessarily reflect US EPA policy.*

1481 A Robust Protocol for the Differentiation of Stem Cells to a Neuronal-Glial Co-culture to Assess Developmental Neurotoxicity

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There is a high need for animal-free, human-relevant *in vitro* models to assess developmental neurotoxicity (DNT) potential of chemicals. Essential in this major effort is to have reliable protocols that mimic parts of neural development, preferably performed within a short time period. This study presents a robust and reproducible protocol to differentiate human stem cells, both embryonic and induced pluripotent stem cells, into neural progenitor cells (NPC). Within the course of two weeks this is followed by differentiation into a co-culture of a variety of neuronal cells and astrocytes that presents spontaneous network activity. Gene expression of markers typical for cell differentiation were regulated as expected: stem cell marker *POU5F1* decreased dramatically during differentiation, while neural progenitor marker *NEUROG1* peaked at the neural progenitor stage. Neural markers *TUBB3* and *MAP2* increased steadily over time and astroglial marker *GFAP* increased strongly only between the NPC and neural stage. Gene expression results could be confirmed with immunostainings. Gene and protein markers for synapses (*PSD95, SYNPR*) and transporters (*SLC17A6, SLC32A1*) increased in the last stage of differentiation, which was also functionally reflected by spontaneous network activity measured on a multi-well micro-electrode array. The results were reproducible across different banks and cellular origin, and a neural network is formed within two weeks This hESC model can be employed to study the effects of chemicals on the *in vitro* differentiation of human NPC into a neuronal - glia co-culture, representing an essential process in human brain development. The differentiation protocol is reproducible and relatively easy to perform and is relevant for regulatory purposes.

1482 RyR-Active Polychlorinated Biphenyls (PCBs) Cause Neurobehavioral Deficits in Larval Zebrafish

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Despite being banned since the late 1970s, PCBs continue to pose significant risk to the developing nervous system. Early life stage (*in utero* or during infancy) exposure to PCBs is associated with increased risk of neuropsychiatric disorders. A common neuropathologic feature of neuropsychiatric disorders is altered patterns of dendritic arborization in central neurons, and we have previously demonstrated that non-dioxin-like (NDL) PCB congeners alter dendritic arborization in the developing mammalian brain via sensitization of ryanodine receptors (RyR). Structure activity relationships (SAR) of RyR sensitization by PCBs (both NDL and dioxin-like congeners) have been determined using tissues from not only mammalian tissues but also rainbow trout (*Oncorhynchus mykiss*) muscle tissue. However, whether this SAR translates to developmental neurotoxicity (DNT) of PCBs *in vivo* has yet to be tested. To address this gap, we are evaluating the DNT of PCBs 28, 66, 84, 95, 138, and 153 in the zebrafish model. We first confirmed that these PCB congeners exhibited differing RyR activity in zebrafish muscle ranging from negligible (PCB 66) to moderate (PCB 153) to high (PCB 95) activity. We then tested the effects of static embryonic exposure to each of these PCB congeners on photomotor behavior. Enzymatically dechorionated embryos were exposed to varying concentrations (0.1-10 μM) of these six congeners from 6 to 120 h post-fertilization (hpf). Embryos were observed daily by stereomicroscope for mortality and gross malformations and behavioral assessments were conducted at 72, 96, and 120 hpf. The body burden of each PCB was measured by gas chromatography. At the concentrations tested, none of these PCBs caused death or overt teratology. A subset of these PCB congeners altered photomotor behavior in larval zebrafish, and the SAR for PCB behavioral effects mirrored the SAR for RyR sensitization. Quantification of PCB levels in larval zebrafish ruled out the possibility that congener-specific effects on behavior were due to differential uptake of PCB congeners. These data support the hypothesis that RyR sensitization contributes to the *in vivo* DNT of PCBs. *This work was supported by the NIEHS (grants ES014901 and ES011269).*

1483 Iodine Insufficiency Exacerbates Structural Defect in Rat Brain following Developmental Exposure to Ammonium Perchlorate

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Iodine is required for thyroid hormone (TH) synthesis, severe deficiencies in iodine leading to hypothyroidism. As TH is necessary for brain development, maintaining iodine status is especially important during pregnancy. Perchlorate is an environmental contaminant that interferes with iodine uptake and reduces TH production. As such, pregnant women with iodine deficiency (ID) may be particularly susceptible to perchlorate exposure. In this study we examined how dietary ID and perchlorate exposure, alone and in combination, affect serum TH profiles in pregnant rats and their progeny. As a measure of neurodevelopmental insult, offspring were examined on postnatal day 14 (PN14) for the presence of a periventricular heterotopia, a known TH-dependent phenotype within the brain. Naïve female rats were maintained on either an iodine-replete (Control) or ID diet for a minimum of 4 wks; this ID regimen reducing serum T4 by 35%. Animals were bred and on gestational day 6, half of each group was exposed to 0 or 300 ppm perchlorate in the drinking water. Serum T4 in newborn pups was decreased 20% by ID or perchlorate, differences that were ameliorated by PN6. In contrast, serum T4 in offspring of dams exposed to both ID and perchlorate was decreased by 57% in the neonate, with further reductions by PN14. Heterotopia were not observed in pups from ID or control dams. Perchlorate-exposure induced small heterotopia in more than half of the animals examined (6 of 10). In contrast, large heterotopia were observed in all pups (12 of 12) born to dams exposed to the combination of ID and perchlorate. Quantification of heterotopia revealed a severe defect (mean volume of 0.0542 mm^3). These data indicate that neurodevelopmental effects of perchlorate exposure are not only unmasked but greatly exacerbated under conditions of dietary iodine insufficiency, conditions that result in permanent structural defects in the developing brain. *Does not reflect US EPA policy.*

1484 Benzalkonium Chloride Disinfectants Induce Apoptosis and Activate the Integrated Stress Response in a 3D *In Vitro* Model of Neurodevelopment

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Benzalkonium chlorides (BACs) are a class of disinfectants widely used in food processing, health care, and residential settings. Recent studies demonstrated that BAC exposure leads to an increased incidence of neural tube defects *in utero* and increased apoptosis of neural progenitor cells (NPCs) at high concentrations *in vitro*. We previously found that BACs alter cholesterol and lipid homeostasis in cultured neuronal cells and in brains of neonatal mice exposed to BACs *in utero*. However, the effects of BACs on neurodevelopment as a result of altered cholesterol biosynthesis has not been investigated. Here we investigate the effects of BACs on neurospheres, free floating structures of NPCs, which are used as a three-dimensional *in vitro* model of neurodevelopment. We chose a short-chain BAC (BAC-C12) and a long-chain BAC (BAC-C16) to cover the structural and biological effect variations of the BACs. Thus, NPCs were isolated from embryonic mouse brains at embryonic day 13.5-14.5 and cultured in suspension to allow the formation of neurospheres until day *in vitro* 4 (DIV4). The neurospheres were then grown until DIV7 in the presence or absence of various concentrations of BACs ranging from 1 to 100 nM. Consistent with our previous work in a neuronal cell line, we observed potent inhibition of cholesterol biosynthesis at the step of Dhcr7 by the short-chain BAC-C12 at 50 nM, but not by BAC-C16. Interestingly, neurospheres exposed to either BAC decreased in size, which was not observed in neurospheres exposed to AY9944, a known Dhcr7 inhibitor, suggesting that cholesterol biosynthesis inhibition is not responsible for the reduction in neurosphere growth. Using immunocytochemistry, we found that both BACs decreased the number of proliferating cells and increased the number of apoptotic cells. To further explore the mechanisms underlying the similar biological actions of both BACs, we carried out RNA sequencing on neurospheres exposed to each BAC at 50 nM for 24 hours, which revealed activation of the integrated stress response by both BACs, including upregulation of *Trib3* (a mediator of ER stress-related neuronal apoptosis), *Asns* (asparagine synthetase), and *Slc7a11* (a transporter that mediates the intracellular level of cysteine). Overall, these results add to a growing body of literature that BACs may have detrimental effects on neurodevelopment. *Supported by NIH Grants P30ES007033, T32ES007032 and R01HD092659.*

1485 Trace Fear Conditioning in Adult Offspring after Concurrent Perinatal Exposure to Variable Environmental Events and Manganese

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Developmental exposure to variable stressors may alter learning and memory formation in offspring. Our research examines if exposure to nonchemical environmental events during development influences the effects of a concurrent chemical exposure. The current study examined if perinatal exposure to variable nonchemical environmental events and/or exposure to manganese (Mn) in drinking water alters learning after a trace fear conditioning (TFC) protocol. Pregnant Long-Evans rats were exposed to a series of unpredictable environmental events, which have been shown to increase maternal corticosterone levels. These events were presented daily from gestational day (GD) 13 through postnatal day (PND) 9. Groups of control and environmentally manipulated dams were exposed to 0, 2, or 4 mg/mL of Mn in drinking water from GD7 through PND22. Starting at PND97, male and female offspring were trained with a TFC protocol. TFC consisted of pairing a compound light and tone cue followed by a 30 sec. trace period and a foot shock (1mA, 0.5 sec. duration). Five pairing sessions occurred on the training day where all groups displayed reduced movement after each pairing. The next day context learning was assessed by placing rats back into the training chamber and measuring activity without cues. Cue learning was also tested on the day after training by measuring activity in a novel chamber where the light and tone cue were presented. Data suggests that all groups displayed reduced movement indicative of learning during context testing. No significant treatment effects were noted for perinatal environmental manipulations, Mn, or concurrent exposure to both when compared to control offspring motor activity. Ongoing research will look for transcriptional changes in the hippocampus and amygdala of these adult offspring after learning TFC. *This abstract does not necessarily reflect US EPA policy.*

1486 Perinatal Exposure to Propylthiouracil (PTU), but Not DE-71, Induces Heterotopia in the Brain Despite Similar Effects on Serum T4 Levels

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Thyroid hormones (TH) are crucial for brain development and low maternal serum thyroxine is associated with adverse effects in their children. These effects can manifest as lower IQ, delayed motor development and increased risk of neurobehavioral disorders such as autism and schizophrenia. Concurrently, a number of environmental chemicals can interfere with thyroid hormone availability and action, raising concerns that these chemicals can disturb thyroid hormone balance and adversely affect child brain development. Propylthiouracil (PTU) exposure of pregnant and lactating rats decrease serum thyroxine (T4) levels in dams and offspring, and induce a range of effects in the offspring's brain. Here, we show that offspring of PTU-exposed dams had periventricular heterotopia, a congenital malformation of the brain. These are irreversible clusters of ectopic neurons in the corpus callosum, which form, in a dose dependent manner, as a result of reduced perinatal T4 levels. We compared heterotopia formation after PTU exposure with that of two other suspected thyroid disrupters: the UV-filter octyl methoxycinnamate (OMC) and a mixture of brominated flame retardants (DE-71). Only PTU induced heterotopia, even though DE-71 reduced T4 levels in the offspring to a similar degree (~75% reduction). Expression of a battery of thyroid hormone responsive genes was analyzed in the pup cortex as potential biomarkers of concurrent TH action. PTU reduced gene expression in a dose-dependent manner, whereas the low serum T4 levels in DE-71 offspring did not alter expression levels. On the other hand, OMC altered gene expression at a time when there was no change in TH levels. This suggests that circulating serum T4 levels, altered by environmental chemicals, are not necessarily reflecting thyroid hormone action in the brain. In order to protect the fetus and the developing child from thyroid hormone disrupting chemicals, future studies should elucidate both adverse effects and the conditions allowing for effects to eventuate.

1487 A Mouse Neural Organoid Model Generated in Multi-Well Plates on Synthetic Hydrogels for Developmental Toxicity Applications

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There is a vital need to create physiologically relevant *in vitro* models that can predict developmental toxicity mechanisms. As the developing brain consists of multiple cell types including progenitor cells, neurons and astrocytes, all interacting and supporting each other, we propose that an organoid model containing these cells, formed in a developmentally regulated manner would be an important *in vitro* model. A mouse model serves as a good starting point for several reasons: 1) The developmental timing of the mouse brain is rapid and we can achieve organoids expressing complex phenotypes and astrocytes in the course of a four week experiment, and 2) There is ample *in vivo* data publicly available with neurotoxin treatment of mice for comparison purposes. We have differentiated mouse embryonic stem (ES) cells to neural progenitor cells and then plated these on a custom-designed, synthetic polyethylene glycol (PEG) hydrogel substrates in culture conditions designed to enable formation of neural organoids. Organoids at day 20 were characterized using neuronal (βIII tubulin, MAP2, DCX) and astrocyte (GFAP) markers. We have utilized RNA-Seq approaches and gene-set enrichment analysis (GSEA) to investigate the effect of different substrates (for example, our optimized synthetic hydrogel vs. the complex animal-derived material, Geltrex®) in the development of neural organoids and observed that the synthetic hydrogel substrate significantly upregulated biological pathways such as central nervous system differentiation, synaptic transmission, neuron maturation and synapse formation over Geltrex®. In addition, we validated our *in vitro* model using previously published *in vivo* mouse dataset by Lein *et al.* (Nature, 2007) and found that the described neuron marker gene set was significantly upregulated in mouse neural organoids cultured on the synthetic hydrogel. We then tested the model by treating it with a small set of neurotoxic compounds (Chlorpyrifos, Valproate, Lead Acetate and BDE-99) at IC_{50} concentrations for four days. We observed significant higher LDH cytotoxicity in chemical treatments compared to the control. RNA was extracted from organoids for transcriptomic analysis, which is currently in progress. By quantifying the outputs such as change in gene expression and cytotoxicity, our *in vitro* model will aid pathologically- and physiologically relevant predictions of developmental toxicity.

1488 A Tiered Skin Irritation Safety Assessment of a Face Cream Product

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The US Food and Drug Administration (FDA) does not require specific testing to demonstrate the safety of personal care and cosmetic products or their ingredients. Recently, a tier-based assessment was proposed by Cardno ChemRisk to assess the skin irritation potential of personal care and cosmetic products. This safety evaluation framework was applied to two newly formulated face cream products prior to them being commercially available to consumers. The first tier utilized the Organization for Economic Co-operation and Development (OECD) QSAR Toolbox to perform an *in silico* evaluation of the skin irritation potential of the product ingredients. Subsequently, we reviewed ingredient-specific safety reviews from the Consumer Ingredient Review (CIR), European Chemicals Agency (ECHA), and Scientific Committee on Consumer Safety (SCCS) to identify any ingredients with potential skin irritation hazards. For the tier two analysis, the OECD 439 EpiDerm Skin Irritation Test (SIT) was performed to evaluate the skin irritation potential of the face cream product utilizing a reconstructed human epidermis. Tier one results showed that out of the 85 evaluated ingredients, 8 ingredients (BHA, BHT, menadione, methyl miristate, phenoxyethanol, tocopherol, avobenzone, isopropyl palmitate) received an *in silico* structural alert for skin irritation, and an additional 17 ingredients (arnica montana flower extract, carbomer, cetearyl alcohol, lemon peel oil, disodium EDTA, eucalyptus globulus leaf oil, fragrance, isododecane, lavender oil, rosemary leaf oil, sodium lactate, sorbitan olivate, stearic acid, stearyl alcohol, cholesterol, sodium hydroxide, and tetrasodium EDTA) were identified as potentially associated with skin irritation based on previous safety evaluation reviews. However, based on the results from *in vitro* testing (tier two), the face cream product was classified as a non-irritant. This study demonstrates the value of a tiered testing approach for personal care and cosmetic products, as the presence of a potential ingredient hazard does not necessarily constitute a risk.

1489 Incorporation of Particle Deposition and Clearance in the Development of Occupational Exposure Limits (OELs) for Active Pharmaceutical Ingredients (APIs) with Minimal Oral Bioavailability

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Inhalation is the primary route of exposure in the workplace and key in the considerations in setting of an OEL. Conventional assumptions are that the entire dose that is inhaled remains in the lung and can be absorbed systemically. This may be more accurate for small molecules (MW <1 kDa) but is generally not applicable for larger molecules (i.e., proteins or oligonucleotides). The deposition of these biologic-type APIs can influence their ultimate fate and potential pharmacological action either systemically or locally in the respiratory tract. An examination of the deposition of respirable and inhalable particles was modeled to identify a relevant fraction that could then be used in setting OELs. The Multiple Path Particle Dosimetry (MPPD) model (V3.04 2002-2016) was utilized to understand the percent deposition of 1.5, 2.5, 4, 7, 10 and 50 µm mass median aerodynamic diameter (MMAD) particles to the pulmonary (PU), tracheobronchial (TB) and head regions of the respiratory tract under active and at rest conditions. For each region, the highest percentage deposition was identified at approximately 17%, 16%, and 98% for particles with a MMAD of 4 µm, 7 µm and 50 µm, respectively. From a literature review, the percentage of particles not rapidly cleared from the PU, TB, and head regions was estimated to be approximately 100%, 75% and 20%, respectively. Both factors were combined to identify a relevant fraction of 17% (PU), 12% (TB) and 20% (head), which is assumed to have a potential to remain in the lung region long enough to impart pharmacological or toxic effects. These values can be used to estimate a relevant dose to portions of the respiratory tract. For example, for an immunomodulatory drug that targets B-lymphocytes that is present in the PU, TB and head, one could estimate a worst-case relevant fraction of 50%, which would be applied to adjust the inhaled dose in the OEL determination. This would be a dose capable of causing direct lung effects and would be a reasonable point of departure. Understanding the relevant fraction of the total inhaled dose, provides a realistic, if somewhat conservative estimate of the exposure for hazard characterization and risk assessment purposes.

1490 Derivation of a Health-Based Screening Value for Fentanyl on Indoor Surfaces

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Fentanyl is a potent synthetic opioid that is 50-100 times more potent than morphine. Routes of exposure for therapeutic use are transdermal patches, transmucosal lozenges/sprays, and injectable formulations. Oral and transmucosal therapeutic doses in humans begin at approximately 0.0025 mg/kg; transdermal doses range from 0.0125 to 0.1 mg/hr. Increased clandestine fentanyl production operations in residential structures present potential health concerns. The low amount required to induce adverse effects and high dermal/mucosal uptake are concerning for remediation of illicit fentanyl production facilities. In the United States, there is currently one remediation standard which is based on analytical detection limits (California). There are currently no guidelines available to assist stakeholders in determining whether fentanyl is present at levels that could impact human health or to conclude whether cleanup efforts were successful from a health-based perspective. We derived health-protective screening values (HBSVs) for fentanyl on residential surfaces after remediation utilizing surface wipe sampling. Following CalEPA's approach in their methamphetamine remediation derivation, we utilized any effect induced by the drug as an adverse effect and possibly a critical effect. We considered exposure paradigms including incidental ingestion and dermal contact with dust residues present on indoor surfaces for different age groups. Inhalation exposures were not considered significant due to fentanyl's extremely low volatility and data from similar cleanups (methamphetamine houses) indicating re-suspension of material was unlikely post-remediation. Exposure modeling results indicate that dermal absorption from floor contact followed by hand-to-mouth behavior of young children (6 months to less than 2 years) was the most important factor for establishing a screening value for surfaces. We found no published acceptable daily intake levels; in lieu of this, provisionary advisory levels produced by the EPA for oral exposures (0.00086 mg/kg-d) and dermal clinical studies for transdermal exposures were used as the bases for our derived HBSV. The resulting derived HBSV for fentanyl on surfaces was 0.0002 mg/100 cm^2.

1491 Derivation of Health-Based Exposure Limits (HBEL) for a Drug Substance, Naproxen Sodium

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HBELs, such as a Permitted Daily Exposure (PDE) and an Occupational Exposure Limit (OEL), are routinely developed for active pharmaceutical ingredients (APIs) by pharmaceutical companies, but are seldom published by expert panels or regulatory agencies. APIs differ from other chemicals in that APIs are designed to impart a physiological change in an individual and there are generally robust API-specific human data sets. Specific guidance for API HBEL development was published by International Society of Pharmaceutical Engineers and European Medicines Agency. Naproxen sodium, a propionic acid derivative and an approved non-steroidal anti-inflammatory drug (NSAID), was selected because the HBEL values for this API are of high importance since naproxen is used frequently as a surrogate for industrial hygiene (IH) performance testing. The purpose of this work is to demonstrate the appropriate incorporation of clinical and other data into an OEL and PDE derivation and provide clear justification for each point of the assessment. To develop the OEL and PDE, the publically available non-clinical and clinical pharmacodynamics, pharmacokinetic and toxicological data were collected and evaluated. Both the critical effect for humans (the lowest clinical dose) and a nonclinical study [lowest-observed-adverse-effect level (LOAEL) for developmental toxicity] were selected as points of departure. Adjustment Factors, aligned with the above guidance, including F1 (interspecies), F2 (intraspecies) with a calculated chemical specific adjustment factor, F3 (subchronic to chronic), F4 (severity of effect) and F5 (LOAEL to no-observed-adverse-effect level [NOAEL]) were considered and then applied to the POD. A bioavailability factor (α), accumulation factor (S) and modifying factor (MF) are described. The assumptions leading to determination of HBEL values are compared and contrasted. While the data set for deriving the PDE and the OEL are the same, the HBEL values differ based on differential considerations for protection of worker and patient health. In general, the OEL does not equal PDE divided by 10. Overall, an HBEL incorporates all available data; while nonclinical data serve a valuable comparative purpose, as clinical data are usually more conservative and more relevant for the human risk assessment involved in determination of HBELs.

1492 Human Health Risk Assessment for Pulegone, a Carcinogenic Flavor Chemical in Mint- and Menthol-Flavored Electronic Cigarette Liquids and Smokeless Tobacco Products

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In 2018, FDA banned six flavor chemicals from addition to food, due to their carcinogenicity in animals. One of these compounds, pulegone, was detected in US-marketed mint/menthol-flavored e-liquids and smokeless tobacco products (SLT). In contrast, the tobacco industry minimized pulegone content in menthol cigarettes. Since FDA proposed to exempt mint/menthol-flavored e-cigarettes from sales restrictions, the human health risk associated with pulegone in these products needs to be assessed. Margin of Exposure (MOE), a risk parameter used by FDA for assessing carcinogenic risk of food additives, was calculated by dividing the FDA-provided no-significant-adverse-effect-level (NOAEL) of pulegone at which no carcinogenicity was reported (13.39mg/kg-bw/d) by the Estimated-Exposure-Dose (EED) for humans from use of mint/menthol-flavored e-cigarette or SLT products. EEDs were based on pulegone concentrations in products and daily amount consumed by users (light user-5ml e-liquid/10g SLT; moderate-10ml e-liquid/20g SLT; heavy-20ml e-liquid/30g SLT). Pulegone MOE was compared between mint/menthol-flavored e-cigarettes, SLT products and menthol cigarettes. The carcinogenicity risk is inversely proportional to MOE, with a threshold of 10,000; thus, MOE's below 10,000 require risk-mitigating interventions. Depending on daily consumption rates, MOEs ranged between 325-6,012 for e-cigarettes and 549-1,646 for SLT, all significantly below the threshold of 10,000, indicating increased risk for users. Predicted pulegone exposure in e-cigarette users was 44-1,608 times higher, and in SLT users 126-1,319 times higher than in menthol cigarette smokers. Users of mint/menthol-flavored e-cigarettes and SLT are exposed to pulegone levels significantly higher than the FDA considers unacceptable for food intake. Exposures from e-cigarettes and SLT are several orders of magnitude higher than from combustible menthol cigarettes. FDA is advised to prioritize reduction of pulegone levels to mitigate carcinogenic risk before endorsing mint/menthol-flavored e-cigarettes for harm reduction. Inaction will further increase health risk for users from continued exposures to carcinogenic pulegone.

1493 Application of the Dermal Sensitization Threshold Concept to Chemicals Classified as High Potency Category for Skin Sensitization Assessment of Ingredients for Consumer Products

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Skin sensitization evaluation is a key part of the safety assessment of ingredients in consumer products, which may have skin sensitizing potential. The dermal sensitization threshold (DST) concept, which is based on the concept of the thresholds of toxicological concern, has been proposed for the risk assessment of chemicals to which skin exposure is very low level. There is negligible risk of skin sensitization if a skin exposure level for the substance of interest was below the reactive DST which would protect against 95% of protein-reactive chemicals. For the remaining 5%, the substance with the defined knowledge of chemical structure (i.e., High Potency Category (HPC) rules) needs to be excluded from application. However, the DST value for chemicals classified as HPC has not yet been proposed. In this study, we calculated the 95th percentile probabilities estimate from distributions of skin sensitization potency data of 116 HPC sensitizers from the expanded data set using the publicly available LLNA data from the OECD Toolbox database and the national toxicology program interagency center for the evaluation of alternative toxicological methods (NICEATM) database. As a result, we derived a novel DST for chemicals classified as HPC (HPC DST) of 1.5 μg/cm^2. This value presents a useful for the default approach for unidentified substances in ingredients considering, as a worst case scenario, that the unidentified compound may be potent skin sensitizer. Finally, we developed a novel risk assessment workflow incorporating the HPC DST.

1494 Utility of Subchronic Transcriptional Points of Departure for Agrochemical Chronic/Carcinogenic Risk Assessment

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Although transcriptional data have been used to inform toxicity modes of actions, use of these data for human health risk assessment is not yet widely accepted. It was hypothesized that a subchronic transcriptome-based point of departure (POD) derived from target organ (e.g., liver) effects would approximate the apical endpoint POD from a chronic and cancer bioassay. In separate studies, male rats were dosed orally with four pesticide active ingredients: triclopry, inatreq, sulfoxaflor and pronamide for 13 weeks. Liver and/or kidney transcriptomes were analyzed by RNA sequencing, and a biological effect POD (BEPOD) was derived using BMDExpress. All apical endpoints with treatment-related effects observed in previously conducted rat and mouse cancer bioassays were analyzed using benchmark dose (BMD) methods to establish noncancer and cancer PODs. A comparison of rat BEPOD values with rat apical PODs for the noncancer and cancer apical endpoints showed the apical PODs and BEPODs were within 2-fold, specifically, 1.33, 1.69, 1.13, 1.62-fold, whereas mouse apical PODs and rat BEPODs were within 10-fold, i.e., 8.80, 0.15, 2.10 and 5.23-fold for four agrochemicals. Furthermore, the liver BEPOD was within 10-fold of the apical POD even for agrochemicals without apical liver effects. Our results are consistent with previous findings using environmental chemicals and suggest BEPODs from rat subchronic studies might be used to estimate apical PODs from rat and mouse chronic and cancer bioassays. To this end, a framework is proposed to incorporate shorter-term BEPODs into overall weight of evidence to estimate a chronic and cancer bioassay POD, thus enabling the potential to conduct a chronic/carcinogenic risk assessment without performing a chronic and cancer bioassay.

1495 Glyphosate Risk Assessment to Assess Proposition 65 Requirements for Pesticide Applicators and Construction Workers: Risk Communication Case Study

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Glyphosate, the active ingredient in Roundup™, has had wide-spread use for many decades, but its listing under California's Proposition 65 law has raised the need to assess warning requirements. Risk communication challenges are heighted by widespread media coverage of large litigation judgements and controversial scientific opinions. To assess the need to warn and provide informed messages regarding potential hazards related to a large herbicide application and subsequent construction project, exposure to herbicide applicators applying glyphosate at the maximum recommended application rate for turf, post-application exposure to construction workers, as well as to surrounding residents and recreators, were quantitatively assessed. A unique site-specific construction scenario was evaluated involving removal of turf and topsoil, traffic on treated soil and burying treated media as fill. For applicators, exposure levels, non-cancer hazard, and potential cancer risks were calculated using EPA's Occupational Pesticide Handler Exposure Calculator for two application scenarios including mixing, loading, and applying herbicides using a hand sprayer, and using a tractor ground boom. To evaluate construction worker exposures, the EPA Regional Screening Level Calculator for construction worker activities was used to assess exposure. Relevant exposure pathways included soil ingestion, dermal contact, and inhalation of resuspended dust. Dead grass and soil samples were collected three days following the final application, and the levels of glyphosate in treated turf were approximately 200 mg/kg. The calculated exposures for all scenarios were at least 2,000-times lower than the No Significant Risk Level (NSRL) (1,100 μg/day). The non-cancer hazard index for construction workers was 0.009, well below the target level of 1, and that for applicators was 0.02. These results indicated that warnings were not required, and risk communication messaging effectively lessened concerns of potential health risk and liability for future litigation.

1496 Volatile Organic Compounds Measured in US Indoor Residential Air from Smoking and Nonsmoking Homes and Implications for Public Health

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Volatile organic compounds (VOCs) are commonly found in the indoor residential environment from major sources such as tobacco smoke, building materials, and household consumer products. Factors that can influence the extent of exposure in a home include presence of a basement, attached garage, ventilation, and building age. A total of 22 studies were evaluated and data for indoor air concentrations and personal exposures to 14 common VOCs were pooled across a wide range of United Stated residential homes. Sampling times reported in the studies ranged from 2.5 hours to 6 months. VOC concentrations were compared between smoking and non-smoking homes and evaluated against available acute and chronic governmental health benchmark levels for non-cancer endpoints. Specifically, acute (8-hour) and chronic Agency of Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs) and US Environmental Protection Agency (EPA) (8-hour) acute exposure guideline levels (AEGLs). Weighted averages of indoor area samples ranged from 0.72 μg/m^3 to 44.79 μg/m^3 for all VOCs. Weighted averages of personal samples ranged from 0.53 μg/m^3 to 41.29 μg/m^3. Seven VOCs that are common constituents of cigarette smoke were 1.3 to 3.3 times higher in the indoor air of smoking versus non-smoking homes. In contrast, indoor concentrations of chloroform were twice as high in non-smoking homes, potentially due to alternative sources in the home. The indoor concentration of toluene was not different between smoking and non-smoking homes. The weighted average concentration of each VOC was between 2 and 49,000 times lower than acute health benchmark levels, and 2 to 1,440 times lower than chronic health benchmark levels, except for formaldehyde and benzene. The weighted indoor average of formaldehyde in non-smoking homes was 23 μg/m^3, 2.35 times higher than the ATSDR MRL of 9.8 μg/m^3. Similarly, the weighted average indoor concentration of benzene in smoking homes was 1.17 times higher than the respective ATSDR MRL (11.17 μg/m^3 vs. 9.57 μg/m^3). The analysis of the data suggest that most indoor VOC exposures experienced in residential homes will not pose a risk to human health with respect to acute and chronic non-cancer effects.

1497 Application of a Multifaceted Read-Across Approach in the Determination of an Oral Reference Dose for Cyclopentanone

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General population exposure to cyclopentanone occurs through its use as an intermediate in the synthesis of nylon and rubber materials found in drinking water systems. Cyclopentanone is also found in nature, in foodstuffs, and is commercially used in fragrances and flavoring ingredients. An oral risk assessment was needed to establish allowable drinking water levels. Cyclopentanone exhibits low acute oral toxicity with an acute oral LD_{50} ≥1179 mg/kg. Repeated-dose data on cyclopentanone were limited to two non-guideline inhalation studies and a developmental study in rats. Deficiencies in study design or reporting precluded use of these studies for derivation of criteria; however, outside of a finding of mitral cell degeneration, no treatment-related adverse effects were reported. Due to lack of standardized, repeated-dose toxicology studies for cyclopentanone, a read-across approach, using cyclohexanone as a surrogate, was used to determine an oral reference dose (RfD). The selection of cyclohexanone as a surrogate for cyclopentanone was supported based on a weight of evidence approach including the application of QSAR software as well as comparison of available toxicity data, physical-chemical properties, and metabolic pathways. The main metabolic pathway for cyclopentanone, cyclohexanone and other cyclic ketones in general, involves reduction to the secondary alcohol followed by glutathione conjugation and, to a lesser extent, sulfur containing metabolites prior to urinary excretion. From the available repeated-dose toxicity data for the surrogate, cyclohexanone, a critical effect of reduced body weight gain (30-35%) was identified from a two-year drinking water study in F344 rats. The resulting $NOAEL_{HED}$ of 119 mg/kg-day served as the point of departure. Using a total uncertainty factor of 100x (3x interspecies, 10x intraspecies, 3x database), an oral RfD of 1 mg/kg-day was determined for cyclopentanone. The resulting Total Allowable Concentration in drinking water was 8000 μg/L.

1498 Health Assessment of Etocrylene Present in Paints and Coatings

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Etocrylene (CAS number 5232-99-5), a UV energy-absorbing stabilizer commonly used in paint and coating formulations, was identified by chemical analysis (Fourier transform infrared spectroscopy and gas chromatography/mass spectroscopy) to be responsible for a hazy coating observed on the surfaces of a particular brand of cabinetry doors. We assessed the risk of adverse health effects in consumers from exposure to etocrylene on surfaces of cabinetry doors. To determine a maximal exposure to etocrylene from cabinet doors, we considered: 1) the amount of etocrylene "dust" on a standard sized panel of cabinet door (6 mg on a 256 in^2 cabinet door), 2) the average cabinet door surface area of a large-size home (150 ft^2), 3) 100% exposure level, 4) 100% absorption and bioavailability of exposed etocrylene, and 5) the average body weight of a child (18.6 kg) and adult (80 kg). Based on the above assumptions, an individual adult and child would be exposed up to 6.3 and 27 mg/kg body weight, respectively, in a large home. Our review of the literature found very limited studies on the health effects of etocrylene. There is no peer-reviewed literature on the health effects of etocrylene on humans. There is a report of human test subjects exposed to 5% (weight/volume) solution of etocrylene in corn oil via a patch/epicutaneous test that observed no sensitization reactions in test subjects. The health effects of etocrylene have been investigated in a limited number of *in vitro* and animal *in vivo* studies (with exposure levels of at least 1 g/kg body weight). Results of these studies indicate etocrylene is not a sensitizer, mutagenic, irritating to the eye and skin, or a reproductive or developmental toxicant. The no observable effect levels (NOELs) from the reviewed *in vivo* and *in vitro* studies were used as the basis for a margin of exposure calculation. Results indicate the margins of exposure associated with the exposure assumptions outlined previously are about 160 for an adult and 37 for a child. Margin of exposures of this magnitude based on NOELs and upper limit exposure scenarios indicate that no adverse health effects were expected from the levels of etocrylene present on cabinetry doors.

1499 Optimized Safety Assessment of Chemical Sensitizers

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Small chemicals can induce tolerance or sensitization following dermal exposure. Activation of dendritic cells (DC) is an important step in the induction of skin sensitization, and the chemical's potential for DC activation can be evaluated *in vitro* using THP-1 cells, as surrogate DC, in the human cell line activation test (h-CLAT). However, the DC response to chemicals in the skin environment is also modulated by chemical-exposed keratinocytes. We found that THP-1 cells show an increased response (upregulation of activation markers CD86 and/or CD54) to sensitizing chemicals when exposed in coculture with HaCaT keratinocytes. In the past, combining results of a blind study in COCAT (cocultured activation test) with earlier results (in total 26 skin sensitizers) found that all 26 sensitizers were correctly identified, offering a highly accurate hazard prediction. In this study, we applied our optimized COCAT protocol and tested additional 30 molecules, consisting of 50 % fragrances. The fragrance molecules comprised different LLNA classes (ECETOC - Technical Report No. 87, 2003), 1 molecule classified as strong, 4 as moderate and 5 as weak. Concentration-dependent responses and determination of the lowest concentration needed to reach positivity (ECΔ) for CD86 and/or CD54 were received for 80 % of tested molecules, as for some molecules there were interferences. Applying our established protocol, a chemical is tested from 7.8 to 4000 μM and is considered a sensitizer if in ≥ 2 out of 3 runs at least one marker reaches its threshold for positivity (ΔMFI ≥ 10.8 for CD86 or ΔMFI ≥ 300 for CD54). Concentrations inducing positivity varied between 101 μM for trans-2-hexenal and 3932 μM for anisylalcohol, offering a dynamic range to grade sensitizers according to their potency. Comparing our results with available LLNA data found Diethylenetriamine to be categorized false-negative in COCAT, while hazard was correctly predicted for 20 of 21 testable sensitizers. These data underline the superior capacity of COCAT for hazard identification of skin sensitizers.

1500 Exposure to Environmentally Relevant Concentrations of Hexavalent Chromium Does Not Induce Ovarian Dysfunction in Mice

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Exposure to hexavalent chromium [Cr(VI)] in drinking water has been reported to adversely affect ovarian follicles in mice. Exposure to high concentrations of Cr(VI) (>=250 ppm) for 20 days was reported to decrease follicle counts, whereas exposure to 5 ppm for 90 days is reported to cause structural anomalies in follicle membranes examined by electron microscopy. Effects at >=250 ppm Cr(VI) were likely confounded by overt toxicity, whereas the effects at 5 ppm were subjective and not quantified in any way. Nevertheless, the purported structural effects in electron micrographs currently serve as the basis for a maximum allowable dose level (MADL) derived by the state of California under Proposition 65 (8.2 µg/day) even though there is limited evidence that exposure to higher levels of Cr(VI) affect reproductive health in rodents. To address these findings, we exposed B6C3F1 mice to 0.1 to 150 ppm Cr(VI) in drinking water for 90 days in a GLP-compliant study to examine the potential for effects on follicle health. As expected, water consumption and bodyweight were reduced in the two highest exposure groups due to poor palatability. Counts of small, medium, large, and total follicles were not altered in any exposure group, nor was the percentage of follicular atresia altered. No evidence of morphological changes were apparent in any treatment group by light microscopy. There were no microscopic changes in the adrenal glands, pituitary gland, thyroid/parathyroid glands, mammary glands, vagina, or corpus and cervix uteri. With the exception of the pituitary gland, there were no treatment-related effects on organ weights. The NOAEL for follicle toxicity (150 ppm) leads to a MADL of ~2900 µg/day. Although this MADL is more scientifically defensible than the current MADL, the current study calls into question whether a MADL should be based on follicular toxicity given the lack of effects at 150 ppm and the frank toxicity that is likely associated with exposure to higher Cr(VI) concentrations.

1501 Decreasing Uncertainty in Existing MRL Values and Derivation of New MRL Values in the Absence of New Health Effects Data via Use of Updated Methodologies

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Updates of ATSDR's minimal risk levels (MRLs) are most often prompted by the availability of new health effects data. However, recent profile updates have demonstrated the importance of reviewing existing MRLs against current risk assessment methodologies, even if newer data have not been identified. Most notably, the continued development and refinement of benchmark-dose (BMD) modeling methodologies provide more accurate dose estimates for point-of-departures (PODs) used in MRL derivation. Additionally, use of human equivalent concentrations (HEC) as PODs instead of administered concentrations decreases the uncertainty in the MRL (e.g. reduces the total uncertainty factor [UF] needed to derive MRLs). Here, we present three profiles with revised/new MRLs derived solely based on updated methodologies: 1,2-dichloropropane (1,2-DCP), endrin, and bromodichlormethane (BDCM). For 1,2-DCP, the acute and intermediate MRLs (both existing and provisional) are based on nasal lesions reported at all tested concentrations by Nitschke and Johnson (1983) and Nitschke et al. (1988), respectively. For the acute inhalation MRL, use of a HEC value as the POD instead of administered concentrations decreased the POD from 100 ppm to 1.8 ppm and decreased the interspecies UF from 10 to 3. For the intermediate inhalation MRL, use of the $BMDL_{HEC}$ value of 0.05 ppm as the POD instead of the administered LOAEL value of 15 ppm removed the LOAEL-to-NOAEL UF of 10 and decreased the interspecies UF from 10 to 3. For endrin, the existing profile did not derive an acute oral MRL because ATSDR considered the available POD for the critical effect (neurotoxicity) a serious LOAEL. At the time, ATSDR did not have BMD modeling capabilities – which allow estimations of PODs below serious LOAELs – and use of a serious LOAEL as a POD for MRL derivation goes against ATSDR practices. However, with current BMD methods, ATSDR identified a BMDL value of 0.06 mg/kg/day for neurotoxicity, which was then used for MRL derivation. For BDCM, ATSDR revised the acute oral MRL using updated BMD methodology, which identified a previously unavailable, better-fit model for the data. This decreased the POD from 10 mg/kg/day, based on the multistage 2-degree model, to 7.15 mg/kg/day, based on the dichotomous hill model. Collectively, these examples demonstrate how the application of updated methodology alone produced more accurate and protective MRLs.

1502 Ethylene Oxide: Cancer Evidence Integration and Dose-Response Implications

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Ethylene oxide (EtO) is a highly reactive chemical used as a sterilizing agent and a feedstock for producing other chemicals. IARC and EPA classified EtO as a known human carcinogen. EPA concluded, "Overall, confidence in the hazard characterization of EtO as 'carcinogenic to humans' is high" and derived a published inhalation unit risk (IUR) value (3×10^{-3} per (µg/m^3 EtO). The IUR is calculated from a supralinear dose-response (DR) model based on transformed relative risk estimates from two epidemiological studies. However, neither of these studies demonstrated any increased risk of breast cancers or lymphohematopoietic malignancies (LHM) compared to the general population. EPA also derived IURs from three chronic rodent bioassays; these IURs indicate a 100-fold lower potency than the IUR based on human data. We examined the EPA's reliance on a mutagenic mode of action (MOA) to establish that EtO is a human carcinogen and considered possible DR patterns. Toxicological studies and studies of early effect biomarkers in animals and humans indicated no clear relationship between EtO and LHM or breast/mammary cancers. Only one chronic study of leukemia in female Fisher rats demonstrated a clear monotonic DR; however, this strain is recognized to have a high background rate of leukemia and may not be an appropriate model for human leukemogenicity. Overall, the animal data were inadequate to define the actual DR shape or predict tumor response at very low doses with any confidence. Per EPA policy, a linear low-dose extrapolation approach should be used for a mutagenic MOA. DR analysis of selected key events in the MOA in humans and animals indicated high uncertainty in DR shape. There is no indication that a low-dose extrapolation approach other than linear is justified. Further, comparison of the range of no observable adverse effect levels (NOAELs) for the key events from humans and animals revealed substantial range overlap and did not suggest a 100-fold difference in cancer potency reflected by EPA-derived IURs. We conclude that the epidemiology does not demonstrate that EtO is a human carcinogen and that the toxicological data do not support deviation from linear low-dose extrapolation or a two order of magnitude interspecies difference in cancer potency. Thus, the current EPA IUR for EtO clearly overstates human cancer risks.

1503 Factors Influencing Dose-Response Assessment (DRA) for Monovalent Inorganic Phosphate Compounds (MIP)

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Phosphorus (P) is ubiquitous in nature and an essential constituent of all living organisms. Humans are exposed to P naturally in the diet and by widespread use of MIP, such as orthophosphate (H_3PO_4) and its sodium and potassium salts, in fertilizers, food additives, consumer products, and water treatment. Animal data, and more limited human data, suggest the occurrence of adverse health effects associated with excessive P intake and/or high levels of P in serum. DRA for MIP faces challenges: 1) Physiological levels of P are tightly regulated. Homeostatic mechanisms maintain serum P levels within a narrow range, even in the presence of wide variations in intake. As a result, studies that evaluate health effects associated with serum P levels may not be useful for DRA. 2) Dietary intake of phosphates is difficult to quantify, and the bioavailability of phosphates varies widely with source (10-30% plant, 40-60% animal, 80-100% MIP). Most of the dietary intake studies do not account for different sources of P in the diet, and none provide estimates of intake from MIP. Therefore, dietary intake studies may not be useful for DRA. 3) Human colonoscopy preparation studies provide a known dose of defined material (sodium phosphate) to subjects but include exposure for only one day. There is considerable uncertainty in extrapolating from effects of a single day to subchronic or chronic exposure. 4) Animal toxicity studies are abundant, but few of the available studies include information on concurrent calcium intake, a key determinant of phosphate bioavailability, limiting the candidate points of departure for RfD derivation. 5) P is an essential nutrient. Doses that are below nutritional needs may lead to deleterious effects. The recommended daily intake (RDI) for P may not be a lower bound on the RfD for MIP because the RDI includes P from less bioavailable organic sources in addition to inorganic sources (MIP). 6) A significant proportion of the US population may exhibit conditions or characteristics that increase their susceptibility to phosphate toxicity. For example, chronic kidney disease (CKD) impairs the body's ability to excrete excess P, leading to hyperphosphatemia. People with CKD are typically prescribed phosphate binders and/or instructed to reduce their intake of dietary P below the values recommended for healthy persons. FDA recommends against the use of MIP to treat constipation in infants and children under 5, unless under physician supervision, due to their enhanced susceptibility.

1504 Development of Reproductive Toxicity Criteria for 4-Vinyl-Cyclohexene and Its Metabolite Vinyl Cyclohexane Diepoxide

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4-Vinyl-cyclohexene (VCH) is a 1,3-butadiene dimer that primarily serves as an industrial intermediate in, for example, polymer synthesis; it may be present in various products as a residual, potentially resulting in consumer exposure. CYP450-mediated bioactivation of VCH to epoxides results in ovotoxicity, with vinyl cyclohexane diepoxide (VCD) being the most potent epoxide in terms of ovarian follicular depletion. Mice are more sensitive to VCH's ovotoxicity compared to rats following inhalation or oral exposure, reflecting their greater rate of epoxide formation. Subchronic to chronic dermal or oral exposure to VCD results in uterine atrophy, ovarian follicular atrophy, and ovarian tubular hyperplasia in B6C3F1 mice. Sprague-Dawley rats also show loss of ovarian follicles following intraperitoneal injection of VCD. Based on these data, both VCH and VCD are included on the State of California's Proposition 65 list as female reproductive toxicants. However, no safe harbor Maximum Allowable Dose Level (MADL), which serves as the basis for quantitative Proposition 65 regulatory compliance determinations, has been developed for either chemical. We developed an MADL for VCH based on a Texas Commission on Environmental Quality (TCEQ) risk assessment that identified a 250 ppm inhalation-route point of departure (POD), equivalent to 44.6 ppm after adjusting for less-than-continuous exposure, for ovarian atrophy in B6C3F1 mice. Inter-route extrapolation from the adjusted exposure concentration in air to a corresponding daily dose, using default female mouse body weight and inhalation rate values, yielded a VCH dose of 321 mg/kg/day. This daily dose was multiplied by the OEHHA-established female human body weight of 58 kg and then divided by 1,000, in accordance with OEHHA guidelines, to obtain a MADL of 18.6 mg/day for VCH. For VCD, we performed a dose-response assessment to identify the POD upon which to base its MADL. Across VCD studies in rats, mice, and non-human primates, the highest NOAEL that is lower than the lowest LOAEL is an i.p. NOAEL of 5 mg/kg/day from a 4-week rat study that identified ovarian atrophy. As was done for VCH, the POD for VCD was multiplied by the 58 kg default female body weight and divided by 1,000 to obtain an MADL of 290 µg/day. These MADLs will aid risk assessors to limit potential reproductive harm from VCH or VCD present in consumer products by supporting California Proposition 65 regulatory compliance determinations.

1505 Exposure to 1,4-dioxane above the Metabolic Saturation Threshold Induces a Mitogenic Key Element in the Mouse Liver Cancer Mode of Action

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The US EPA has completed two risk evaluations of 1,4-Dioxane (1,4-DX) carcinogenicity, an IRIS assessment in 2013 and the recently published, draft TSCA risk evaluation in 2019, where human cancer risks related to rodent liver tumors were characterized using the default non-threshold model. The available evidence for the rodent liver tumor response better aligns with a threshold-dependent, tumor promotion Mode of Action (MOA) but early key events (KEs) in this MOA have not been well defined for the mouse model. To address this, we conducted a study in female B6D2F1/Crl mice. Mice consumed drinking water containing 0, 40, 200, 600, 2,000 or 6,000 ppm 1,4-DX (targeted at 0, 10, 50, 150, 500 and 1,500 mg/kg/day, respectively) for up to 90-days. Blood levels of 1,4-DX and its major metabolite, 2-hyrdoxyethoxyacetic acid (HEAA), were determined to characterize the time course of absorption and clearance of 1,4-DX. Indices of hepatic proliferation, histopathology, mRNA analysis and BrdU incorporation were used to characterize the time course and magnitude of a proposed KE (proliferation) resulting from exposure to 1,4-DX. There was a dose and time dependent increase in blood levels of HEAA with a supralinear increase in 1,4-DX blood concentration with exposure to 6,000 ppm demonstrating saturation of metabolic clearance. There was a corresponding, and previously unreported, increase in liver weight and BrdU incorporation in the absence of cytotoxicity and genotoxicity indicating a direct mitogenic stimulus triggered by 1,4-DX accumulation. These data advance the understanding of early KEs in the MOA for 1,4-DX and confirm previous reports of a threshold MOA based on metabolic saturation and accumulation of parent compound.

1506 Probabilistic Risk of Decreased Levels of Triiodothyronine following Chronic Exposure to PFOS and PFHxS in Drinking Water

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High concentrations of perfluorinated alkyls (PFAS) have been found in drinking water and in human serum due to contamination from industrial production sites or extensive use of firefighting foams. In many places the identified levels are found to be above the guidance levels and there is a need to characterize the risk in terms of probability for adverse health effects. We performed an integrated probabilistic risk assessment combining the serum levels of perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) measured in residents of a heavily contaminated area in Sweden with data from a subchronic toxicity study where Cynomolgus monkeys were exposed to PFOS. The critical outcome was defined as a 10% decrease in total triiodothyronine (T_3) hormone levels. The 5th and 95th percentile of human serum concentrations for the residents (n = 1,845) were 63.3 - 830.9 ng/mL (PFOS) and 45.0 - 795.5 ng/mL (PFHxS). A benchmark dose analysis was employed to describe the dose-effect relationship in animals and extrapolation distributions were used to estimate the corresponding human benchmark dose for decreased T_3 levels. The median probability of critical exposure (PoCE), following a combined exposure to PFOS and PFHxS, was estimated to be 2.1% (90% C.I. 0.4% - 13.1%). A separate gender based analysis showed that the risk was mainly distributed among women (PoCE = 3.9%, C.I. 0.8% - 21.6%), with an estimated PoCE for men of 0.08% (C.I. 0.02% - 2.85%). As the initial deterministic risk characterization, based on the same key study and similar level of adversity, indicated an exposure close to the tolerable daily intake of 150 ng/kg/day, this study exemplifies a shift of narrative following the use of a probabilistic approach as a tool for risk assessment.

1507 Derivation of No-Significant-Risk-Levels (NSRLs) for 4-Vinylcyclohexene and 4-Vinylcyclohexene Diepoxide

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4-Vinylcyclohexene (VCH) and 4-vinylcyclohexene diepoxide (VCD) are listed on the State of California's Proposition 65 List as known to the State to cause cancer and reproductive toxicity. The cancer listings are based on the results of National Toxicology Program (NTP) two-year bioassays. Oral dosing with VCH led to dose-related increased incidences of skin and preputial gland tumors in male rats, lymphoma and lung tumors in male mice, and ovarian and adrenal tumors in female mice. Dermal dosing with VCD resulted in dose-related increased incidences of dermal tumors in each sex of each species and ovarian tumors in female mice. Preneoplastic ovarian lesions were detected in mice dosed with VCH and VCD. Industries are obligated to comply with Proposition 65 labeling requirements and drinking water discharge prohibition for products sold in California, unless they are able to demonstrate that VCH and VCD levels in their products result in consumer exposures below specific safe harbor levels, specifically No Significant Risk Levels (NSRLs) for carcinogens. As the State of California has not published NSRLs for VCH or VCD, we derived NSRLs based on NTP tumor data in accordance with California Office of Environmental Health Hazard Assessment guidelines. Additionally, as VCD is a metabolite of VCH, we sought to compare the carcinogenic potential of these chemicals. The Carcinogenicity Potency Project Database (CPDB) identified a TD50, the dose producing a 50% tumor incidence, of 106 mg/kg/day for VCH based on the increased ovarian tumor incidence identified for mice in the NTP study. We linearly extrapolated the TD50 to a probability of one excess cancer case in an exposed human population of 100,000 (10^-5 cancer risk), resulting in a NSRL of 123 µg/day for VCH. As the CPDB identified no TD50 for VCD, we performed benchmark dose (BMD) modeling via the US EPA's BMD Software to derive BMDs for the tumors detected in the NTP rodent bioassays of VCD. The resulting CSFs were scaled to human equivalent doses (HEDs) using body weight scaling. The most conservative HED from models with appropriate statistical fit corresponded to combined skin tumors in male rats. A VCD cancer slope factor (CSF) was derived from the BMD for skin tumors, and a NSRL of 4.9 µg/day was calculated using this CSF and a 10^-5 cancer risk. These NSRLs can be used as part of risk and safety assessments to evaluate compliance with Proposition 65 requirements for consumer products containing VCH and/or VCD and sold in California.

1508 Risk Analysis of Current Ozone Levels in New Delhi, India

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In today's world, air is full of toxic gases resulting in adverse effects. In the last decade, increased combustion of igneous fuels results in progressive change in the atmospheric composition. Ozone is the most steady and unmanageable air pollutant in urban air and an ideal candidate causing damage to respiratory system as previously reported. The site of damage after prolonged ozone exposure is the alveolar region. Levels as low as 50 ppm can result in decreased levels of ascorbic acid, uric acid and glutathione, resulting in pulmonary obstruction, bronchial hyper responsiveness and airway inflammation. While EPA reports that over 70 million people in the US reside in areas not meeting the one-hour EPA ozone standard, present day estimate of mortality in India owing to lung damage such as COPD due to ozone exposure is about 12000 people (India census 2011). In this study, we assessed the risk using FMEA by utilizing the available regional data in the metropolitan city of New Delhi on ozone and produced risk assessment for the current ozone levels in the Indian sub-continent and determined the at-risk populations. Finally, our data highlights the emerging role of ozone exposure as an air pollutant in the Indian sub-continent.

1509 Assessment of the Relevance of Toxicological Findings in the Development of an Oral Reference Dose for GenX

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Detection of GenX, an alternative to the long-chain per- and polyfluoroalkyl substances (PFAS), in water sources near production facilities has led to the need for development of toxicity criteria protective of oral exposure. Assessment of available toxicity data (i.e., chronic, subchronic, reproductive, and developmental toxicity studies in rats and mice) demonstrated that although the liver is a primary target organ in both rats and mice, GenX has been reported to cause other effects such as reduced fetal and pup body weight, reduced pup survival, and altered mammary gland development. The strength of evidence and relevance for developmental effects was assessed, including an analysis of confounding effects such as reduced maternal feed intake in high dose groups and transcriptomic evidence for enrichment of altered glucose metabolism and gluconeogenesis. Liver effects in mice were determined to be consistent with peroxisome proliferator-activated Receptor-α (PPARα) activation, including increased liver weight, liver hypertrophy, and increased peroxisomal enzyme activity. Re-evaluation of liver tissue from previously conducted studies in mice (2010) using more current histopathological diagnostic criteria demonstrated that lesions previously diagnosed as 'single cell necrosis' were in fact apoptosis. This was subsequently corroborated by immunostaining for activated caspase-3 as well as transcriptomic pathway enrichment analyses. GenX was also found to increase mitosis in the liver at the same doses as apoptosis, which is consistent of rodent-specific cell cycle changes induced by PPARα activators. The involvement of PPARα and absence of liver necrosis, fibrosis, and inflammation indicate that the mouse liver lesions have limited relevance for risk assessment. Overall, the analyses support development of an RfD based on liver lesions observed in rats exposed for 2 years. Modeling these lesions using both frequentist and Bayesian benchmark dose (BMD) models, resulted in deterministic and probabilistic RfD values for GenX of 0.02 and 0.01 mg/kg/ day, respectively. Incorporation of the more conservative of the two RfD values in standard equations for development of a maximum contaminant level goal yielded an acceptable concentration in drinking water of 70 ppb.

1510 Risk Assessment of Manganese Exposure for Pica Child: Application of PBPK Modeling

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Steel slag aggregate used as road cover can contain up to 3% manganese (Mn). Although Mn in slag is not readily released for absorption into the body if incidentally ingested (33% bioaccessibility), we assessed the potential for neurotoxicity associated with a hypothetical child displaying pica behavior. Soil pica is when a young child ingests higher levels of soil than is expected, on the order of 1,000 to 5,000 mg/day or more. Information regarding the prevalence of pica behavior is limited, but it is considered most prevalent among children 1 to 3 years of age, and it is estimated that approximately one-third of children ingest more than 10 grams of soil 1 or 2 days per year. Since no acute toxicity criteria for ingestion of Mn exist, previously published physiologically-based pharmacokinetic (PBPK) models for Mn were used to predict potential uptake of Mn into the brain globus pallidus for two hypothetical exposure scenarios, in addition to daily Mn exposure via diet and inhalation of ambient air. Exposures for a 2-, 3- and 5-year old child were modeled for slag intakes of: 1) 1,000 mg/day for 5 consecutive days, or 2) 10,000 mg twice in one year, occurring 6 months apart. The model predicted that both the peak concentration (Cmax) and Area Under the Curve (AUC) of Mn in the globus pallidus were below the level of concern associated with neurotoxicity. Specifically, the model predicted Cmax ranged from 0.46 to 0.80 ug/g, with AUCs of 208 to 791 ug/g/h, and half-lives of 1-3.5 days. Cmax increased from 9% to 48% but rapidly return to baseline concentrations. The PBPK results suggest that the target brain Mn exposure from pica behavior would be well under the levels associated with neurological effects (AUC > 6000 ug/g/h or Cmax >7 ug/g from chronic intake) and more importantly, the increase is transient. In summary, pica behavior, although probably quite unlikely with steel slag, would not result in the intensity and duration of Mn exposure that could cause neurological effects in children, and these methods can be applied in other Mn health risk assessments.

1511 Occupational Risk Assessment for the New Oxidative Hair Dye 2-methoxymethyl-p-phenylenediamine: Improved Protection of Hairdressers from Allergy Induction

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Occupational exposure of hairdressers to the strong sensitizers p-phenylenediamine (PPD) and toluene-2,5-diamine (PTD) through oxidative hair dyes has been associated with the development of allergic contact dermatitis. The PPD and PTD replacement 2-methoxymethyl-PPD (ME-PPD) combines equally excellent hair coloring performance with a moderate a skin sensitizing potency. Quantitative risk assessment (QRA) was applied to comparatively assess the occupational risk of hairdressers to become sensitized to either PPD or ME-PPD containing hair dye products. Hairdressers' hand exposure to ME-PPD was determined by collecting samples following typical routine hair color treatments in commercial salons in Germany using a hand rinse method and HPLC analysis. Published data were available for PPD and PTD. Daily hand exposure concentrations were derived by considering assessment factors for wet work, uneven hand exposure and inter-individual variability for professionals. QRA was conducted by comparing daily hand exposure with the sensitizing potency defined as the No Expected Sensitization Induction Levels (NESIL). For ME-PPD the hairdresser hand exposure level is approximately 100 fold below the NESIL while hand exposure to PPD is 2.7 fold below the NESIL, respectively. In conclusion, QRA reveals that the use of ME-PPD in oxidative hair colors significantly reduces the occupational risk of allergy induction for hairdressers compared to PPD and PTD.

1512 Toxicological Risk Assessment to the Typical Constituents of E-cigarettes in China

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Electronic nicotine delivery systems, which is also called e-cigarettes, are devices used for vaporizing e-cigarette smoke liquid that comprises with nicotine, propylene glycol, glycerine, and flavourings. Previous studies suggest that chemical constituents between the aerosol produced by heating e-cigarette liquid and the traditional cigarette smoke are quite different. Specifically, the composition and the release of harmful constituents from e-cigarette are significantly lower. However, there is still a lack of obvious evidence that the risk of human exposure by e-cigarette is seriously reduced when compared with traditional cigarette because of little research. In this work, we randomly select 26 e-cigarette liquids from China market to analyze their harmful constituents and the corresponding release amounts, including nicotine, glycerine, propylene glycol, formaldehyde, acetaldehyde, 2,3-butanedione, butyraldehyde, Propionaldehyde, crotonaldehyde, 2-butyraldehyde, acetone, and acrolein. The technology of risk assessment we used is a scientific evidence-based analytical process. Results indicate that compounds like nicotine, glycerin, propylene glycol, formaldehyde, and acetaldehyde can be detected in all samples, with the release amounts under the level of 0.04, 1.95, 1.47, 0.59, and 0.33 g/puff, respectively, while the detection rates of propionaldehyde, crotonaldehyde, and 2-butyraldehyde were more lower, which can be detected by 19.2%, 11.5% and 11.5% samples, respectively. Both 2, 3-butanedione and butyraldehyde were not detected in the aerosol. The detection rates of acetone and acrolein are 23.1% and 38.5%, with the release

amounts of 0.13 and 0.23 g/puff. We also performed field investigation on 517 e-cigarette consumers living in Beijing, Shanghai, Guangzhou, and Shenzhen cities of China. The smoking parameters were recorded by CReSS Pocket. Results showed that about 95% consumers used the e-cigarette containing tank systems, which can be added the liquid. Their usage frequency of e-cigarettes is about 84.6 puff/day, with a capacity around 87.2 ml/puff. The lifetime average daily dose of each constituent (LADD) from aerosol was calculated by LADD=(C×IR×ED)/(BW×LT), which is then used to calculate HQ by HQ =LADD/RfD, where RfD is the reference dose. According to IRIS of US Environmental Protection Agency (US EPA), the RfDs of formaldehyde, acetone, and acrolein are 2×10^{-1}, 9×10^{-1} and 5×10^{-4}mg/(kg·d) respectively. The finally obtained HQs for formaldehyde, acetone, and acrolein are 4×10^{-3}, 2×10^{-4}, and 6×10^{-1}, respectively, indicating that these hazards might not be expected to be a threat to the public health.

1513 Oral Administration of Hexavalent Chromium Did Not Increase Mutant Frequency in the Small Intestine of *gpt* Delta Mouse

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WHO/IARC categorizes hexavalent chromium (Cr[VI]) compounds as Group I human carcinogens. In animal model studies, exposure to the Cr[VI] compound, sodium dichromate, via drinking water for 2 years increased the incidence of tumors of the oral mucosa and tongue in rats and of the small intestine in mice. However, the *in vivo* mutagenicity of Cr[VI] compounds in target organs must be evaluated prior to assessing the cancer risk they pose. We examined the *in vivo* mutagenicity of Cr[VI] in the small intestine, the target organ of tumorgenicity, by using a transgenic mouse gene mutation assay. In our studies, male *gpt* delta mice received sodium dichromate dihydrate in drinking water at tumorigenic doses (85.7 or 257.4 mg/L for 28 days or 8.6, 28.6, or 85.7 mg/L for 90 days). Compared with that in vehicle control mice, *gpt* mutant frequency in the small intestine of Cr[VI]-exposed mice was not increased significantly in either the 28- or 90-day study; in contrast, 28-day oral administration of potassium bromate at a dose of 2.0 g/L as a positive control significantly increased the mutant frequency to three times higher level than that of vehicle control. Our results indicate that Cr[VI] compounds are not mutagenic in target organs, such as the small intestine, at tumorigenic doses, suggesting that non-mutagenic mechanisms, such as cytotoxicity, contribute to the tumorigenicity of Cr[VI] compounds.

1514 Hazard Assessment of Diacetyl and Structurally Related Diketones—A Read-Across Approach

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Diacetyl (2,3-butanedione) is a volatile organic compound with a strong buttery flavor that is commonly used as food flavoring ingredient and in electronic cigarettes. Concerns were raised regarding respiratory disorders (Popcorn Lung) such as bronchiolitis obliterans linked to occupational pulmonary exposure to diacetyl vapors and use of electronic cigarettes. In this read-across study, we investigated the use of new approach methodologies to characterize and differentiate the hazard of diacetyl and other structurally related diketones. For this purpose, three structurally similar groups (α, β and γ diketones) which most likely have different modes of action were included, together with 2 negative compounds aliphatic aldehydes, which do not show any inflammatory response in the available rodent *in vivo* studies. Primary human bronchial epithelial cells (PBECs) were isolated from tumor-free lung tissues from four donors and differentiated into mucociliary epithelial cells at air-liquid interface (ALI) conditions. The cells were exposed to the case study chemicals under (ALI) conditions using the P.R.I.T.® ExpoCube® device for 1h once or repeatedly on three consecutive days. Cellular viability was measured by LDH-leakage and barrier function by measuring the transepithelial electrical resistance (TEER) 24h after the final exposure. Exposure concentrations ranged from 100 to 1840 ppm (diacetyl) and from 50 to 5000 ppm (other diketone analogues). Chemical-induced transcriptomic responses were investigated utilizing targeted RNAseq with the Templated Oligo Detection Assay (TempO-Seq) based on a 3347 gene panel. TempO-Seq analysis revealed up or down regulated differentially expressed genes (DEGs) in a dose and exposure time dependent manner. Analysis of the gene expression patterns indicated that some of these diketones may share a similar mode of action. Translational analyses were carried out to link these *in vitro* data to relevant adverse human outcomes like pulmonary fibrosis and inflammation. *Acknowledgement: This project received funding from the European Union's Horizon 2020 research and innovation program (grant agreement No 681002).*

1515 Using Study Evaluation to Inform Evidence Integration for Chemical Risk Assessment: Application in a Systematic Review of Hexavalent Chromium Male Reproductive Outcomes

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In the application of systematic review for chemical risk assessment, study evaluation is used to identify the strengths and weaknesses of the evidence base in a consistent and transparent manner. This can be used to inform evidence integration by identifying factors that may affect the reliability and interpretability of the results. Here, we describe how this principle was applied in a systematic review of the health effects of hexavalent chromium [Cr(VI)], focusing on animal toxicology studies that evaluated male reproductive outcomes. A literature search for studies on the health effects of Cr(VI) was conducted by searching three online databases (PubMed, Web of Science, Toxline) through May 2018, and supplemented by manual searches of key references, gray literature searches, and "backward" searches to identify articles cited by included studies, reviews, or prior assessments by other agencies. Twenty-three animal toxicology studies that examined the effects of Cr(VI) exposure on the male reproductive system were identified. These studies were evaluated by at least two independent reviewers for reporting quality, risk of bias, and sensitivity using a domain-based approach, and were rated as *high* confidence, *medium* confidence, *low* confidence, or *uninformative*. Of these, eight studies were considered *uninformative* due to serious flaws and were excluded. Four studies had no notable concerns and were considered *high* confidence, and eleven had significant concerns across multiple study evaluation domains and were considered *low* confidence. Whereas the *high* confidence studies found that the male reproductive system appeared unaffected by Cr(VI) exposure, the *low* confidence studies found a range of effects including decreased sperm quality and quantity and altered hormone levels. Taken together, the reliability of the observed effects in animals was considered to be compromised by risk of bias and sensitivity concerns; therefore, these findings contributed very little to the overall weight of evidence for male reproductive outcomes. *The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.*

1516 Systematic Review of the Potential Respiratory Carcinogenicity of Metallic Nickel in Humans

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The inhalation of dust containing certain nickel compounds has been associated with an increased risk of lung and nasal cancers in occupational studies of workers who process or refine sulfidic nickel ores and are exposed to relatively high levels of mixtures of water-soluble, sulfidic, oxidic, and/or metallic forms of nickel. Nickel compounds are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC), while metallic nickel is classified as a Group 2B carcinogen. IARC has identified metallic nickel as a priority substance for review within the next 5 years. We conducted a systematic review of the potential carcinogenicity of metallic nickel, focusing on cancers of the respiratory tract, which is the target organ for tumors induced by nickel compounds. We evaluated the quality and risk of bias of the relevant epidemiology, experimental animal, and *in vitro* mechanistic studies using the National Toxicology Program's Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool. We then used a systematic review protocol based on the OHAT approach to critically assess whether metallic nickel should be considered a human respiratory carcinogen. Our evaluation of the epidemiology evidence indicates that there is no increased risk of respiratory cancers in workers exposed predominantly to metallic nickel. In addition, cross-classification analyses of worker cohorts exposed to multiple forms of nickel indicate that there is no evidence to suggest that metallic nickel exposure resulted in increased respiratory cancer risk. Animal evidence indicates that metallic nickel does not increase the incidence of respiratory tumors in rodents exposed by inhalation. The mechanistic evidence indicates that metallic nickel can induce DNA strand breaks *in vitro*, but is not mutagenic, suggesting that any DNA damage induced by metallic nickel is repaired. Nevertheless, *in vitro* studies are limited in value, as they bypass normal clearance mechanisms and do not consider the low bioavailability of nickel ions from metallic nickel particles in the cell nucleus. After integrating

the evidence from all study types, and applying a standard framework for causality, we concluded that any relationship between metallic nickel exposure and respiratory cancer in humans is not likely to be causal.

1517 Comparison and Analysis of Discrepancies among Commonly Used Cramer Decision Tree Methods in Toxtree Software

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The threshold of toxicological concern (TTC) is a practical tool to screen and estimate safe human exposure levels to compounds lacking adequate data for safety evaluation. In the absence of compound-specific toxicological data, approaches, such as the Cramer Classification Scheme, can be utilized to estimate an appropriate exposure level. The Cramer Classification Scheme is a 33-question decision tree used to classify the potential toxicity of non-carcinogenic compounds with limited toxicity data based upon a curated dataset of oral NOAELs. Divided into 3 classes, the Cramer decision tree scheme categorizes organic chemicals as possessing either low (Class I), moderate (Class II), or high (Class III) potential for toxicity. Although it is possible to manually assess compounds using the decision tree, Toxtree software has developed an automated software approach which implements the Cramer decision tree scheme. Two other decision tree methods, Cramer rules with extensions and the revised Cramer decision tree, are also available and utilized by many scientists in order to predict probable toxicological risk of compounds, although standardized regulatory guidance regarding which tree is most appropriate is currently unavailable, especially considering current applications of the TTC to a variety of chemicals and potential human exposure routes and regimens. The Cramer rules with extensions includes 5 additional questions as derived by Munro et al. (1996) to overcome possible misclassification of compounds. By contrast, the revised Cramer decision tree is a shortened scheme which combines several Cramer questions and incorporates additional industry-derived data on metabolism, toxicity and biochemistry of compounds within the dataset. Recently, analysis of several compounds using these various Cramer schemes elicited conflicting data. Evaluation and comparative analysis among examples using the original, extended, and revised versions of the Cramer decision tree are presented in this poster. By presenting these chemical examples with conflicting Cramer classification, it is possible to gain a better understanding of potential risk associated with each compound and influence of classification scheme on prediction outcomes. Analysis of these chemical examples assists in determination of the most appropriate Cramer decision tree to select with regards to the most adequate, safe human exposure level.

1518 Identifying Target Organs and Candidate Contaminants Based on Adverse Outcomes following Subchronic Oral Exposure in Rats to Contaminated Soil Extracts from a Pesticide Manufacturing Site

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Soil sample extracts from a legacy contaminated site were examined using an effects-directed approach. The study aimed to identify target organ toxicities after twice-weekly oral gavage of 3 different soil extracts (vehicle control and 0.1% of each extract in polyethylene glycol) in male Sprague Dawley rats (n=10/group). After 28 days, a significant increase in blood markers of inflammation was seen in rats exposed to all 3 extracts compared to control. A significant reduction in cholinesterase activity was observed in plasma, but not brain from rats exposed to extract A compared to control. A significant increase in hepatic ethoxyresorufin-o-deethylase activity was also observed after exposure to extracts A and B compared to control. Oxidative stress was detected in the brain and kidney tissues after exposure to extracts B and C, respectively. Acute tubular necrosis was seen in rats exposed to all 3 extracts. Candidate causative agents include organochlorine, organophosphate/carbamate insecticides or their metabolites. Kidney damage and systemic inflammation are clear targets, but some risk may arise from brain oxidative stress. Actual human risk is unclear and must be assessed in combination with potential exposure levels.

1519 Systematic Evidence Mapping of Inorganic Mercury Salts—Mercuric Chloride, Mercuric Sulfide, and Mercurous Chloride

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Systematic evidence mapping (SEM) approaches are increasingly being used to aid in scoping and problem formulation for chemical assessments. In brief, SEMs use systematic review methods to identify the relevant literature and characterize the amount and type of evidence available for an assessment. Through this process, key data gaps and areas of scientific complexity are also identified. The IRIS Program has begun to routinely conduct SEMs as part of its problem formulation activities to promote transparency in the early stages of assessment development. The use of specialized software applications makes production time for SEMs relatively rapid, weeks to several months for most chemicals. This methodology was used to identify relevant literature on the three inorganic mercury salts (mercuric chloride, mercuric sulfide and mercurous chloride) currently under development as IRIS assessments. First, Populations, Exposures, Comparators, and Outcomes (PECO) criteria were developed to outline the evidence considered applicable to address the specific aims of the assessment. Following the literature search from three different databases (PubMed, Toxline, and Web of Science), studies were sorted in SWIFT Review, a text mining work bench tool, using a predetermined list of health outcomes (cancer, cardiovascular, developmental, endocrine, gastrointestinal, etc.) and evidence streams (human, animal, *in vitro*). In addition to screening title and abstract and full-text against the PECO criteria, studies containing supplemental material that may be potentially relevant to the assessment were tracked in DistillerSR. Following full-text review, study designs and results were summarized in a structured format and visualized in Tableau software. The SEM approach identified several oral subchronic and chronic animal studies for both mercuric chloride and mercuric sulfide for further study quality evaluation. No oral studies on mercurous chloride were found. A pool of mechanistic studies, tagged as supplemental, were informative for hazard characterization and linking biomarkers to apical effects. In conclusion, these results will be helpful for consideration of alternative approaches, such as bioavailability and read-across, for use in the derivation of reference values for other data-poor mercury salts.

1520 Determining Mutagenicity of Different Chemicals Using Whole Genome Sequencing of Pooled Clones

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Major advancements have been made over the last decade in next generation sequencing (NGS) technologies and their applications in genotoxicity assessments. An initial disadvantage of NGS is the inability to distinguish somatic mutations from sequencing errors, which can occur every 10^4 bases. To overcome this and accurately identify induced somatic mutations by NGS we utilized a novel approach of performing whole genome sequencing (WGS) of pooled clones. Using WGS of pooled clones for evaluating mutagenicity of a compound subverts the inherent mutation bias of the Ames test toward limited types of mutations and its need for multiple tester strains. The pooled clone approach nullifies the sequencing error-rate disadvantage of NGS and increases number of the targeted base-pairs by simultaneously sequencing of multiple genomes per sample. The Ames test strain, *Salmonella* typhimurium (TA 100), was cultured and treated with different doses of Benzo[a] pyrene (B[a]P), Aristolochic Acid (AA), *N*-ethyl-*N*-nitrosourea (ENU), and 2-Nitrofluorene (2-NF). All these chemicals are mutagenic in the Ames test. ENU, AA, and 2-NF exhibited significant cytotoxicity, resulting in dose-dependent reductions in colony forming efficiency. WGS was performed on pooled samples of five clones for each dose, as well as for vehicle controls, to identify mutations. Treatment with these mutagens resulted in significant mutation induction in a dose dependent manner. The approach also effectively detects mutation signatures specific to each mutagen. Different types of mutations including base-pair substitutions, insertions, deletions, etc. could be visualized and compared using WGS data. We show here that the WGS of pooled clones could be used as a less cumbersome and cost-efficient method for mutation detection.

1521 Significance of Toxicological Interaction Studies

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Sponsor: G. Guillot, EUROTOX

Risk assessment for mixtures of chemicals requires to investigate the presence of potential interactions between those chemicals. This is usually done by assessing how experimental toxicological mixture data depart from the model of Loewe additivity (*Effect of combinations: mathematical basis of problem*, Arch. Exp. Pathol. Pharmakol. 114, 1926). Several recent scientific studies propose to perform this task using an *ad hoc* method known as Model Deviation Ratio (MDR) method. More recently, the first official European regulatory document for the study combined exposures recommends the use of the MDR method (EFSA Scientific Committee et al. *Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals*. EFSA Journal, 2019). We show here that the MDR method is not calibrated, it leads to erroneous decisions and that the results reported lately in studies using the MDR are likely inaccurate. In contrast, we show that a classical statistical technique known as likelihood ratio test provides accurate results, including when sample size are close to the minimum required by OECD guidelines. We show also that the variance of the MDR can be evaluated by simulations and we explain how it allows us to use MDR when no other experimental are available, a situation typical of meta-analyses.

1522 Next-Generation Risk Assessment (NGRA) for Cosmetic Safety Assessment: A Case Study

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Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis-driven approach integrating new approach methodologies (NAMs) to ensure the safety without animal data. We have applied an *ab initio* tiered NGRA framework (Berggren et al., 2017) based upon ICCR principles (Dent *et al*., 2018) to a hypothetical safety assessment of a consumer product - 0.1% coumarin in a face cream. For the purpose of evaluating the use of NAMs, existing animal and human data for coumarin were excluded from the safety assessment. In Tier 0, applied dose exposure estimates for coumarin were determined and an internal C_{max} (0.004 µM) was defined using a physiologically based kinetic (PBK) model. *In silico* chemistry predictions (ToxTree, OECD Toolbox, DEREK) gave alerts for skin sensitisation and genotoxicity. A skin allergy risk assessment was conducted using Tier 1 *in vitro* data for coumarin (DPRA, KeratinoSens, h-CLAT and U-Sens) and a point of departure (PoD) derived using a published Skin Allergy Risk Assessment (SARA) defined approach (Reynolds *et al*., 2019). We conclude a low risk of inducing skin sensitisation in consumers exposed tocoumarin at 0.1% in a face cream. For the systemic toxicity risk assessment, a battery of Tier 1 *in vitro* NAMs were used; genotoxicity (ToxTracker®), receptor-mediated and immunomodulatory effects (Eurofins Safety44 screen and BioSeek® respectively), and non-specific pathways/general bioactivity (ToxCast data, cell stress panel [WC1] and high-throughput transcriptomics (HTTr) in HepG2, HepaRG and MCF7 cells) to which a novel statistical Bayesian approach was applied to the dose-response data. Cell stress and HTTr assays utilising 3D cultures of HepaRG cells were used for Tier 2 refinement. The PoDs from the *in vitro* assays demonstrating a dose response were plotted against the calculated *in vivo* exposure (C_{max} with associated uncertainty) in order to calculate a margin of safety (MoS). The results indicate that coumarin is not genotoxic, does not bind to any of the 44 receptors or show any immunomodulatory effects. The most sensitive PoDs were the inhibition of carbonic anhydrase inhibition assay (Ki= 3-9 µM) and MAO-A and B (AC50= 12-19 µM). The predicted 95th percentile of plasma C_{max} (0.004 µM) was lower than all PoDs. This large MoS indicates that systemic exposure to coumarin following application of 0.1% in a face cream would be of low risk. This case study provides additional data on the value of integrating exposure science with computational modelling and *in vitro* bioactivity data for non-animal, next generation safety assessments.

1523 A Novel Dose-Response Framework with Quantitatively Integrated MOA Information

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In the framework of chemical risk assessment, dose-response assessment first quantifies the relationship between dose and critical health effects, and then extrapolates the biologically/statistically significant dose (i.e., point of departure) to a reference dose for regulatory use. Typically, the POD for carcinogens is the benchmark dose (BMD) or its statistical lower bound (i.e., BMDL). From a conservative perspective, US EPA suggests that linear extrapolation should be used as a default approach to derive cancer slope factor which may be employed to estimate cancer risk at a given dose. If sufficient evidence of mode of action (MOA) can support a conclusion that the dose-response relationship is nonlinear in the low-dose region (i.e., to confirm that the agent is non-mutagenic), an oral reference dose (RfD) or an inhalation reference concentration (RfC) will be calculated instead. However, RfD/RfC lacks the utility to quantify the risk of adverse effects in support of probabilistic risk assessment. Therefore, this project aims to develop a novel framework of dose-response assessment for carcinogens with sufficient data to characterize its MOA. TCDD is used as an example of Aryl hydrocarbon receptor (AhR) mediated MOA for liver tumor to demonstrate the proposed methodology. There are three major steps in the framework: (1) key events along the pathway of TCDD-induced liver tumors are identified by literature review, and the dose-response data are extracted; (2) the BMD method is applied to derive the critical dose level that activates each key event; (3) a pathway dose-response relationship is quantified to estimate the dose level that can induce the key event considered as critical/irreversible in the MOA. As a result, a series of events are identified along the pathway of TCDD-induced liver tumors, including genomic expression changes, enzyme and protein inductions, cellular homeostasis changes, hepatopathy, hyperplasia and tumors. A critical dose of 55 pg/kg/day is estimated by defining the hypertrophy occurred around 31 weeks as the sentinel event for TCDD-induced hepatocarcinogenesis. By quantitatively integrating human variability, this value can be used to derive a risk-specific reference dose. Rather than using a specific health effect to derive a POD in the current dose-response assessment framework, the new approach not only considers the mechanism of tumor formation, but also probabilistically quantifies the dose level that may induce a defined critical key event. This approach may fundamentally unify the linear and nonlinear methods before extrapolating to a human reference dose.

1524 Proposal for Use of *In Vitro* Bioaccessibility Data When Methods Validated Using Animal Models Are Unavailable

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Use of relative bioavailability adjustments (RBA) for lead and arsenic in soil is common in site-specific risk assessment since US EPA approved *in vitro* methods for assessing relative bioavailability of these metals in soil based on validation in animal models. When other metals are evaluated at contaminated sites, exposure estimates are unadjusted for relative bioavailability. The resulting risks are likely overestimated because toxicity values used in estimating risk are typically derived using much more soluble chemical forms of the metal compared to their forms in weathered soil. Use of RBA to refine the risk assessment process would reduce uncertainty in exposure estimates and increase confidence in risk management decision-making. For a site in the western US, *in vitro* bioaccessibility (IVBA) data were collected for metals in 24 soil samples (<149 µm size fraction) for which *in vitro* methods have not been validated using animal models to produce RBA regression estimates: Al, Sb, Cd, Cr, Co, Fe, Mn, Ni, Tl, and Va. The mean IVBA results for Sb, Cd, and Mn were 0.33, 0.82, and 0.52, respectively, and mean results for the remaining metals were 0.20 or below. More specifically, IVBA values for four metals were less than 0.10 (Cr, Fe, Ni, Va) and IVBA values for three additional metals were less than 0.20 (Al, Co, Tl). IVBA also was measured for lead and arsenic, and their respective regression analyses developed in validating the *in vitro* models yielded RBA values lower than the IVBA values for all but the lowest arsenic IVBA result (Pb IVBA=0.1-0.5, RBA=0.06-0.41; As IVBA=0.1-0.5, RBA=0.11-0.43). Comparison of these results to data for metals with validated IVBA methods, i.e., lead and arsenic, shows that the IVBA data are comparable to the RBAs and suggests that use of the unmodified IVBA data (i.e., use of IVBA without adjustment in a regression model) may provide a reasonable estimate of RBA values for use in site-specific risk assessment. Due to limitations in the ability of animal studies to provide RBA estimates for poorly absorbed metals in soil, assuming an RBA of 0.25 to provide an upper-bound estimate of RBA for the seven metals with IVBA values equal to or less than 0.20 is recommended. For Sb, Cd, and Mn, the mean site-specific IVBA values are recommended. Other IVBA/RBA comparison studies reported in the literature also provide evidence that IVBA results are good predictors of RBA, and that using animal models to generate reliable RBA estimates for metals with very low bioavailability is challenging.

1525 Systematic Evidence Mapping of Provisionally Peer-Reviewed Toxicity Value Assessments

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Provisional Peer-Reviewed Toxicity Value (PPRTV) assessments identify and characterize human health hazards and provide an important source of toxicity values for chemicals of concern to the US Environmental Protection Agency's Superfund Program. PPRTV derivation traditionally relies on *in vivo* toxicity databases; however, new approach methods (NAMs) involving expert-driven read-across are being utilized for data-poor chemicals. In the present study, we examined the use and applicability of a systematic evidence map (SEM) approach to aid with the scoping, prioritization and the development of PPRTV assessments for multiple chemicals of interest. A broad-based literature search was conducted using four databases, i.e. PubMed, Web of Science (WoS), Toxline, and Toxic Substances Control Act Test Submissions (TSCATS), followed by title/abstract and full-text screening based on tailored Populations, Exposures, Comparators, and Outcomes (PECO) criteria. Potentially relevant supplemental studies were tagged as mechanistic (genotoxic and nongenotoxic), exposure, absorption, distribution, metabolism, and excretion/toxicokinetics (ADME/TK), case reports or case series, mixtures, studies with no original or useful data, etc. Based on this SEM approach, three lines of assessment development emerged: (1) candidates for traditional PPRTV assessment, (2) candidates for NAM-based read-across PPRTVs, and (3) candidate chemicals where the databases were too limited for the development of an assessment. Through the use of specialized software applications and well-trained staff, the SEMs generally take several weeks to prepare compared to several months by other methods. Results from this pilot study show that systematic review methods can be applied to PPRTVs in an efficient manner and help inform decision-making early in the scoping and problem formulation process.

1526 Variability of Common Toxicological Endpoints, a Comparative Study

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Environmental and occupational public health assessment typically relies on a quantitative framework associated with chemical-specific health guidance values, such as the ATSDR minimal risk levels, and other common toxicity estimators. The latter include the highest-no- and lowest-observed adverse effect levels (NOAELs and LOAELs), benchmark doses, median lethal doses (LD_{50}s), and other. Because each of these endpoints is a measured quantity, they carry a certain level of natural variability. This variability can be of two types: within-study and cross-study variability. The first reflects such factors as the number of subjects, precision of the dose, etc. The second deals with fundamental differences in study design at different labs. Generally, the extent of toxicological endpoint variability is unknown, although a common perception of it does exist. For instance, NOAELs are commonly perceived as less certain quantities than LOAELs. However, such perception often stems from general scientific judgement rather than a rigorous quantitative analysis. In the present work, variability of LOAELs, NOAELs, and LD_{50}s was assessed using information accumulated over years in the toxicological profiles of chemical substances released by the Agency for Toxic Substances and Disease Registry (ATSDR) and other public databases. The variability of each endpoint was estimated using an empirical distribution of sample standard deviations (SDs) of specific endpoint data for each chemical. It was found that the variability of LD_{50}s on the $\log_{10}$ scale was approximately half of that of LOAELs or NOAELs. The median SD of log-transformed LD_{50}s was calculated to 0.26 (95% CI: 0.23 - 0.28), whereas median SDs of log-transformed LOAELs and NOAELs were approximately twice larger: 0.53 (95% CI: 0.50 - 0.58) and 0.57 (95% CI: 0.47 - 0.67), respectively. Note, the calculated SDs of LOAELs and NOAELs were statistically indistinguishable. Indeed, LOAEL and NOAEL represent two adjacent points on the same measured dose-response curve. Therefore, *a priori* expectations of their marked distinctions may be exaggerated. *Disclaimer: The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

1527 Bolstering the Existing Database Supporting the Noncancer Threshold of Toxicological Concern Values with Toxicity Data on Fragrance-Related Materials

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The use of threshold of toxicological concern (TTC) supports the safety assessment of exposure to low levels of chemicals when toxicity data are limited. The Research Institute for Fragrance Materials (RIFM) delivers safety assessments for fragrance materials that result in safe products for consumer use. A major goal for the RIFM safety assessment program is to invest in alternative methods to animal testing for use in assessment of fragrance materials. This includes use of TTC, which provides a pragmatic approach for safety evaluation of fragrance materials in the absence of chemical-specific toxicity data and reduces the need to generate new animal data. To bolster the TTC approach for support of fragrance materials and specifically to strengthen the Cramer class II threshold, the RIFM database was reviewed with a goal of identifying fragrance materials with repeat-dose and developmental and reproductive toxicity data that can be added to the existing TTC databases. The RIFM database identified a total of 476 chemicals that were added to the existing TTC databases. These chemicals from the RIFM database were then individually assigned a Cramer class and 238, 76 and 162 chemicals in Cramer class I, II and III respectively were identified. The RIFM-TTC dataset was then combined with the COSMOS-Federated TTC dataset for a total of 421, 111 and 795 chemicals in Cramer class I, II and III respectively. This further expands the TTC dataset thereby supporting more robust 5th percentile thresholds. This is especially important for Cramer class II: the combined dataset now includes a total of 111 chemicals, which is a significant improvement over the original (Munro) TTC database which only had 28 chemicals in Cramer Class II and the COSMOS Federated dataset which had 40 chemicals. This work confirms the adequacy of the existing TTC values and provides further support for the use of TTC as a tool to conduct safety assessments for fragrance materials. It further opens the future possibility of updating the existing values with more robust TTC values for fragrance and cosmetic materials.

1528 A Method for Comparing the Impact on Carcinogenicity of Tobacco Products: A Case Study on Heated Tobacco versus Cigarettes

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Comparing the harmful health effects related to two different tobacco products by applying common risk assessment methods to each individual compound is problematic. We developed a method that circumvents some of these problems by focusing on the change in cumulative exposure (CCE) of the compounds emitted by the two products considered. The method consists of six steps. The first three steps encompass dose-response analysis of cancer data, resulting in relative potency factors (RPFs) with confidence intervals. The fourth step evaluates emission data, resulting in confidence intervals for the expected emission of each compound. The fifth step calculates the change in CCE, probabilistically, resulting in an uncertainty range for the CCE. The sixth step estimates the associated health impact by combining the CCE with relevant dose-response information. Results: As an illustrative case study, we applied the method to eight carcinogens occurring both in the emissions of Heated Tobacco Products (HTPs) and tobacco smoke. The CCE was estimated to be 10- to 25-fold lower when using HTPs instead of cigarettes. Such a change indicates a substantially smaller reduction in expected lifespan, based on available dose-response information in smokers. However, this is a preliminary conclusion, as only eight carcinogens were considered so far. Furthermore, an unfavorable health impact related to HTPs remains as compared to complete abstinence. Our method results in useful information that may help policy makers in better understanding the potential health impact of new tobacco and related products. A similar approach can be used to compare the carcinogenicity of other mixtures.

1529 Transcriptomic Dose-Response Modeling in >21 Year-Old Archival Mouse Liver Tissue for Risk Assessment

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Gene expression data from short-term *in vivo* studies shows efficacy in quantifying dose-dependent effects that inform and precede toxicity outcomes of regulatory concern. With archival formalin-fixed paraffin-embedded (FFPE) tissue samples, this data can be obtained and related to pathological effects without the need for new animal studies. Advances in technology can help overcome the challenges of isolating and sequencing damaged nucleic acid from old FFPE tissue samples. This study investigated the efficacy of performing transcriptomic benchmark dose modeling (BMD_T, BMDExpress 2.0) on whole genome TempO-sequencing in >21 yr-old paired frozen (FR) and FFPE liver tissue harvested from male mice exposed to 0, 1, 2, and 3.5 g/L dichloroacetic acid via drinking water for 6 d. for which total RNA-sequencing data existed. BMD_Ts calculated from preselected *Cyp4a* targets in FR total RNA sequencing data were 277-284 mg/kg-d, which was ~6.5-fold higher than the IRIS BMD for liver adenoma and carcinoma. BMD_T performed poorly on FFPE total RNA sequencing data due to a 97% loss in gene count signal; however, BMD_T analysis of whole transcriptome TempO-sequencing on the same FFPE tissue only suffered a 40% loss relative to paired FR. Furthermore, DESeq2 analysis of TempO-sequencing data identified 8865 and 16434 differentially expressed genes (DEGs) (FDR<0.05, ±2-fold change) across all doses for FR and FFPE datasets, respectively. Of those DEGs, 5355 were common and 1022 FR and 726 FFPE passed the Williams Trend Test (p-value <0.05) and had BMD_T values ≤ to the highest dose. The median BMD_T value for FFPE TempO-sequencing results was 335 mg/kg-d, 38% higher than paired FR and 16% higher than the FR total RNA sequencing BMD_T results. TempO-sequencing BMD_T for *Cyp4a* target genes in FFPE samples was quite consistent (166-325 mg/kg-d) but not as consistent as the FR (202-225 mg/kg-d). While median TempO-sequencing BMD_T values were still higher than the IRIS BMD, they were within an order of magnitude and calculated from a short *in vivo* exposure (6 d.) unlike cancer endpoints. These BMD_Ts may also be improved with molecular pathway level data, saving time and resources. Our results show very old FFPE tissue samples can be used to obtain quantitative gene expression data and with additional refinement, used to enhance traditional risk assessments. *This abstract does not represent US EPA policy.*

1530 An Interactive Web Application for the Calculation of TD_{50} Values from Carcinogenicity Study Summary Data

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According to ICH M7 guidance, the default approach for calculating compound-specific acceptable intake (AI) values for mutagenic impurities with available carcinogenicity data in pharmaceuticals involves linear extrapolation to a lifetime risk level of 1 in 100,000 from a cancer potency estimate known as the TD_{50}. The TD_{50} is defined as the dose rate which, if administered chronically for a lifetime, halves the probability of remaining tumor-free throughout that period. The Carcinogenic Potency Database (CPDB) serves as a widely-used resource for publicly-available TD_{50} values. The site provides TD_{50} values for 1547 chemical agents calculated from historical cancer bioassays, but it does not include cancer potency estimates from studies published since 2005. Building upon the work achieved by the CPDB project, the non-profit organization, Lhasa Limited, UK has established the Lhasa Carcinogenicity Database (*Toxicol. Res.* 8, 696–703; 2019), which provides TD_{50} values estimated from the same data contained in the original CPDB and, in the future, will include TD_{50} estimates from more recent cancer studies. The statistical methods used by Lhasa Limited to calculate the TD_{50} from summary incidence data were implemented as a script encoded in the statistical package, R. This R-script has been made publicly-available in order to provide a reproducible, documented, and validated method for generation of TD_{50} values. In an effort to give risk assessors a user-friendly way to utilize these statistical methods, we built a publicly available interactive web app (based on the R-script first published by Lhasa Limited) that allows for the calculation of TD_{50} values by fitting the one-hit multistage cancer model to experimental dose-response data. Beyond assessment of mutagenic impurities per ICH M7, calculated TD_{50} values may be useful for the rapid risk assessment of carcinogens in general. An advantage of using the TD_{50} app (as opposed to other more common approaches for estimating cancer potency – i.e. benchmark dose (BMD) modeling, the T25 method, etc.) is ease-of-use. The user is only required to input study summary data; no calculations or BMD modeling expertise are required, thus allowing established cancer potency estimation methods to be utilized by a much wider user base than what was previously possible. Additionally, because the one-hit model is the most conservative of the multistage models, potency estimates generated by the app are often more health-protective than those generated by other methods. Overall, while the app allows users to obtain quick, conservative estimates of cancer potency for use in risk assessments, it is not intended to serve as a replacement for comprehensive dose-response assessment, which includes scientific judgment, fitting experimental data to multiple models, careful evaluation of model fit (especially in the low-dose region), and selection of a "best-fitting" model or model averaging to obtain a point of departure (PoD).

1531 Rodent Short-Term MoA Studies Predict Rodent Tumor Outcome and Point of Departure

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For human health assessment of agrochemicals, carcinogenicity studies in both rats and mice have been used for decades to assess tumorigenic potential of the substances to humans. However, particular concern has been raised about the relevance of the rodent cancer bioassay to assess the human health risk of non-genotoxic carcinogens. Furthermore, when a positive carcinogenicity effect has been identified, human risk assessment may rely on identification of a plausible mode of action (MoA) in animals and establishing if the MoA is relevant to humans. A retrospective analysis of short-term rodent tumor MoA studies and rodent oncogenicity studies on Leydig cell, thyroid and liver tumors was conducted for three agrochemicals (sulfoxaflor, pronamide, nitrapyrin) to examine (1) if tumor NOAELs can be estimated based on the MoA key events NOAELs, (2) if NOAEL from MoA studies, including key events and associated events, are more sensitive than tumor NOAELs. MoA key and associated event endpoints include, but are not limited to, gene expression biomarkers of nuclear receptor activation, cell proliferation, hormonal level, as well organ weight/histopathology. For sulfoxaflor-induced rodent liver, data showed that the MoA key events NOAELs (100 ppm) were concordant with tumor NOAELs (100 ppm). Similar results were observed for pronamide-induced rodent Leydig cell and thyroid tumors NOAELs (200 ppm) and MoA key event NOAELs (200 ppm). Furthermore, pronamide or nitrapyrin-induced liver MoA key events NOAELs (nuclear receptor activation NOAELs = 20 mkd, 75 mkd, increased liver weight/hypertrophy NOAELs = 15mkd, 75 mkd and hepatocellular proliferation NOAELs = 20 mkd, 75 mkd) were protective of liver tumors NOAELs (50 mkd, 125 mkd). In addition to qualitatively predicting potential tumor outcomes and human relevance, these data suggest that a human health-protective point of departure may be established from a short-term MoA study.

1532 Risk Comparison of 1-bromopropane in Production Enterprises and Use Enterprises with Two Toxicological Health Risk Assessment Methods

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The objective was to compare and analyze the risk of 1-bromopropane (1-BP) hazards in production enterprises and use enterprises through two risk assessment methods and to evaluate the occupational health risk. Occupational health investigation and 1-BP detection for workplaces were carried out in three production enterprises in northern of China and in three use enterprises in southern of China. The risk ratings of different posts were assessed by US Environmental Protection Agency (EPA) inhalation risk (EPA assessment model) and Singapore Semi-quantitative Assessment Model (MOM assessment model). The risk classification results of the 2 risk assessment methods were compared and analyzed, based on occupational exposure limits for hazardous agents in the workplace (GBZ2.1-2019) and classification for hazards of occupational exposure to toxicant (GBZ230-2010). The concentration of 1-BP on the positions of reaction, rectification, lavation and packaging were 0.1, 0.9, 1.6 and 0.5mg /m^3 respectively in production enterprises, while on the positions of clamping, cleaning 1 line, cleaning 2 line, and checking were 56.4,29.7,63.4 and 33 mg /m^3 respectively in use enterprises. By the EPA assessment model, all of the positions were evaluated as negligible risk in production enterprises, while the positions of cleaning 1 line and checking were low risk as well as the positions of clamping and cleaning 2 line were medium risk in use enterprises. Through the MOM assessment model, the four positions were negligible risk in production enterprises and all of the positions were medium risk in use enterprises. Current results suggested that 1-BP exposure levels were higher in use enterprises than production enterprises. The EPA assessment model could quantitatively and qualitatively assess the non-carcinogenic effects of chemicals, but the assessment results were low risk and relatively conservative for two positions where the exposure concentration were above the limit. Based on the comprehensive consideration of

both hazard level and exposure level, the MOM model was assessed as more suitable for risk warning than the EPA model for 1-BP occupational health hazard risk assessment in China.

1533 A Systematic Approach to Organize 182 Toxic Air Contaminants by Target Organ: A Risk Assessment Tool

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The Oregon Department of Environmental Quality (DEQ) recently adopted health-risk based regulations for industrial point source emissions of toxic air contaminants. The new regulations require industrial point sources to assess health risks that their emissions may pose to neighbors. The risk assessment process for noncancer effects includes summing hazard quotients for toxic air contaminants that affect the same target organ or organ system. The Oregon Health Authority (OHA) and DEQ developed a spreadsheet that lists target organs and organ systems for all 182 toxic air contaminants regulated under the new regulations. The spreadsheet lists chronic and acute noncancer targets separately. OHA and DEQ created criteria for including a target organ or organ system for each chemical and sought feedback from an external technical advisory committee. The committee had expertise in toxicology, risk assessment, and development of chemical-specific toxicity reference values. OHA and DEQ used summarized information from the US Environmental Protection Agency, Agency for Toxic Substances and Disease Registry, and the California Environmental Protection Agency's Office of Health Hazard Assessment to populate the spreadsheet according to the *a priori* established criteria. For each chemical, the spreadsheet includes the source of information for each target organ and notation about the quality and certainty of information linking the toxic air contaminant to each target organ. The spreadsheet displays a breakdown of uncertainty factors, species tested, and duration of exposure for the critical studies used to derive toxicity reference values for each toxic air contaminant. OHA and DEQ anticipate that this publicly available spreadsheet will be a useful tool for assessing noncancer risks for emissions sources in Oregon and may be useful for risk assessors outside of Oregon as well.

1534 Application of 3R Principles to Dog Studies for Agrochemicals: Building the Case for Eliminating the Dog as a Required Species?

L. Murphy, K. Stebbins, R. Rasoulpour, and C. Terry. *Corteva Agriscience, Indianapolis, IN.*

A core requirement for global pesticide registration is a 90-day toxicity study in a non-rodent species, for which the dog is usually chosen (USEPA 1998, EU 2010/63, OECD 1998, JMAFF 2000). To establish toxicologically relevant dose levels for the 90-day dog study, palatability and 28-day toxicity studies are conducted first. There are no published guidelines for conduct of these preliminary studies, so there is significant opportunity to refine the design and minimize animal use. A combined palatability/28-day study design, using the same animals, is now our standard. The preferred route of exposure is via diet as it is the least invasive route of administration and is more relevant for human health risk assessments. Significant differences in palatability can exist for herbicides and insecticides, and this combined study design considers options and alternatives for each active ingredient. For the herbicide florpyrauxifen benzyl, with no palatability or toxicity issues, the combined study evaluated 500 mg/kg/day in 2 female dogs for 7 days. On day 8, 2 males were offered 500 mg/kg/day for 21 days and 2 dogs/sex/dose were offered 1000 mg/kg/day until day 28. This approach reduces animal use as only two dose levels are tested, and control animals are not needed. Any potential treatment-related effects are compared to pre-exposure measurements of food consumption, body weight and clinical observations on the study animals. The insecticide, sulfoxaflor, presented challenges due to reduced palatability and tolerability with food consumption and body weight effects. Palatability was evaluated using alternatives, such as addition of bacon or peanut butter flavoring, pelletizing the diet, and gelatin capsule administration with the option of supplemental canned diet to encourage food consumption. The combined palatability/28-day study has reduced the use of dogs up to 83% compared to traditional toxicity testing designs and eliminated animal stress indicative of maximum tolerated dose exposures. Further refinements could include consideration of re-homing animals, if termination at study conclusion is not necessary. In addition, animal enrichment opportunities are of critical importance and include consideration of provided toys, minimum daily exercise, and expanded housing space to accommodate regular companionship of group animals. These refinements represent initial, but key steps towards an animal-free toxicity testing future.

1535 Evaluation Framework to Qualitatively Assess the Health Effects of New Tobacco and Related Products

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Many new tobacco and related products (TRP) have emerged on the market, with unknown health risks. Here, we present an evaluation framework containing all factors and relations between them that contribute to the TRP's health effects. As an example, we evaluated the JUUL, a very popular e-cigarette. Factors that determine attractiveness, addictiveness and toxicity of TRP were defined based on previous assessments, literature, and expert discussions, resulting in an evaluation framework. Relations between factors were evaluated, which was supported by literature. Relevant publications on JUUL were used to evaluate all factors. Results: Our framework can be used to identify aspects of TRP that require specific attention for public information or product regulation. In addition, it can gauge attractiveness for specific user groups, while considering exposure. Aspects of concern for JUUL are its attractive and discrete shape, user-friendly prefilled pods, high aerosol nicotine levels, and liquids containing nicotine salts instead of free-based nicotine. Our framework will aid in identifying the key risk factors contributing to increased risk of adverse health effects for a product in a qualitative manner. In case of JUUL, the addictiveness and especially attractiveness are sufficiently high to have a large potential impact on population health due to its contribution to use and hence exposure, even if the amounts of toxicants in the emissions should be lower than for other e-cigarettes. As results can change over time due to changes in use and product modification, market research and monitoring is crucial. Our framework and the tests we recommend can be used for risk assessment of TRP. Since all factors presented contribute (in)directly to the, attractiveness, and addictiveness and toxicity of TRP, policy makers are advised to consider these factors as a possible target for future product regulation. In addition to the identified aspects of concern for the JUUL, we advise to consider regulation of the many JUUL compatible pods, JUUL knock off devices, and e-liquid brands selling high-nicotine products.

1536 Evolving the Use of Respiratory Irritation as a Health Endpoint in Occupational Risk Assessments

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The effects of occupational irritant exposures are of major importance to public health. Approximately 40% of the exposure limit values for agents listed in the current NIOSH Pocket Guide are estimated based on exposure thresholds for respiratory irritation. Respiratory irritation signs and symptoms include rhinorrhea (runny nose), coughing, and airway obstruction and accompanying exposure levels can coincide with sensory irritation responses in other tissues such as stinging and burning in the eyes and skin. Inhalation and dermal exposure to irritants is highly relevant to the use of solvents, catalysts, cleaners, fragrances, and many other agents extensively utilized in workplaces and industrial processes. Despite this, existing guidelines to assess and characterize irritation hazards are limited by inadequate testing methods, poor mode-of-action knowledge, and outdated risk characterizations. NIOSH is responding to these gaps with coordinated efforts across the agency and collaborating partners to meet scientific needs in the following specific areas: 1) Assessing the relationship between sensory irritation responses and traditional respiratory toxicity endpoints in animals (i.e., respiratory depression, airway histopathology, corrosion, inflammatory activation); 2) Investigating the utility of *in vitro* tests for generating irritation toxicity data using primary tissue and cultured cell lines: 3) Developing updated protocols for evaluating irritation toxicity data and integrating existing hazard information to derive exposure limit values, including recommended exposure limits, short-term exposure limits, and ceiling/immediately-dangerous-to-life-and-health (IDLH) values; and 4) Investigating the toxicological mechanisms targeted by irritants and working towards a consistent understanding of the adverse outcome pathways involved. These efforts will address occupational health needs by increasing the prevalence, quality, and utility of irritation toxicity data and improving the efficiency and accuracy of risk assessments for irritation hazards. In addition, these outcomes will be of scientific benefit to consumer product safety, environmental health in industry-adjacent communities, and numerous other public health needs arising from exposure to chemical irritants.

1537 Performance Comparison of BMDL Calculation by the Model Averaging Methods for Quantal Dataset

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At the step of dose-response analysis on the chemical risk assessment, benchmark dose lower bound (BMDL) is increasingly used as an alternative to the NOAEL in order to estimate more appropriate POD for establishing TDI or ADI. Although several statistical models for estimating BMDL are well known, the selection criteria of a model to guide an appropriate BMDL are controversial and there is no international consensus. Recently, as a new concept for BMDL derivation, the model averaging was introduced by the EFSA (PROAST) and the USEPA (BMDS). We have also developed a new averaging method (MA-3) by using frequentist approach like PROAST, and Shao and Shapiro (2018) developed an online model averaging system (BBMD) used Bayesian method like BMDS. Model averaging techniques are said to lead to the most appropriate BMDL, but the consistency of performance on these various systems is unclear. About a hundred dichotomous dose response data sets (mainly from the OECD TG 407 and TG422 studies, and several long-term studies) were used for BMDL calculation. As for calculation software, the BMDS v3.1.1, the PROAST v67, the BBMD and MA-3 were used. Each calculated BMDL was compared to the corresponding NOAEL derived from the actual studies. The BMDL calculated by the BMDS averaged approximately 1.5 times higher than the NOAEL, whereas that calculated by the BBMD was approximately 4.5 times higher. The BMDLs calculated by the PROAST and the MA -3 were approximately 2 and 3 times higher than the corresponding NOAEL, respectively. In case of studies with large numbers of animals per group, differences in BMDL values calculated in these four softwares tended to be smaller. Even though the model averaging methods (especially when using the Bayesian methods) were used, it was clarified that the values of BMDL varied several folds depending on the software used. *Acknowledgment: This study is supported by Research Program for Risk Assessment Study on Food Safety (No. 1801), Food Safety Commissions of Japan.*

1538 Semiautomated Data Extraction Workbench for Environmental Health

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Systematic review, already a cornerstone of evidence-based medicine, gained significant popularity in several related disciplines including environmental health and evidence-based toxicology. A critical, time-consuming process that occurs during systematic review is the extraction of relevant qualitative and quantitative raw data from the text of scientific documents. The specific data extracted differs among disciplines, but within a given domain, certain data points are extracted repeatedly for each review that is conducted. We have recently developed a semi-automated data extraction workbench for use in this context. Our research has focused on three specific goals. First, we are using deep learning to build novel data extraction components for items of interest within the domain of environmental health. Second, we have created web-based software specifically designed for extraction in the context of systematic review. Finally, we have introduced new protocols to standardize the inputs and outputs for data extraction software components. A beta version, currently under evaluation at EPA, includes more than 30 novel data extraction components relevant to environmental toxicology. Performance varies among data types with some tasks inherently more difficult than others. For certain simple data items, like sex of the experimental animal, we achieve F-scores in excess of 95%. For more difficult entities, we were still able to achieve an F-score of 65% or more, when sufficient training data was available. Importantly, the design of our workbench makes it easy to include extraction components developed by other research groups. The workbench currently includes several such components, with new ones added regularly. Because accurate data extraction is a challenging problem, and given that current methods rarely achieve 100% accuracy, we are integrating our methods into a "human-in-the-loop" system that combines machine and human intelligence in a manner that is superior to using either in isolation. The resulting system makes systematic reviews both more efficient to produce and less expensive to maintain, greatly accelerating the process by which scientific consensus is obtained in a variety of health-related disciplines.

1539 Applications of Physiologically Based Pharmacokinetic (PBPK) Modeling in Regulatory Risk Assessment for Pesticides

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Physiologically based pharmacokinetic (PBPK) modeling is widely recognized as a scientifically sound, robust approach for evaluating and characterizing uncertainty in chemical risk assessment by accounting for pharmacokinetic differences across and within species. While the development and application of PBPK models for environmental chemicals, including pesticides, have grown steadily since the 1970s, only a handful of models have been accepted to support regulatory risk assessment due to challenges, such as the difficulty to recruit reviewers with both modeling expertise and risk assessment experience, or the lack of transferability across modeling platforms. Despite these challenges, the number of PBPK models for pesticides submitted to regulatory agencies has risen in recent years. These models have been applied to estimate data-derived extrapolation factors (DDEFs) in lieu of default uncertainty factors when conducting inter-species and/or intra-species extrapolations. In other cases, PBPK models have been used to estimate human points of departure that are specific to exposure routes, durations and frequencies, and life-stages; assess mechanisms of biological responses based on their relationship with dose metrics by linking to a pharmacodynamic model; or investigate the major determinants of pharmacokinetic behaviors for pesticides within the same class. Case studies on triazines, pyrethroids, and carbaryl will be presented to demonstrate these various applications of PBPK modeling analysis that have been accepted by regulatory agencies to support human health risk assessment. In addition to using these examples to highlight how regulatory agencies incorporate kinetic information with computational approaches, lessons learned from reviewing PBPK model submission will also be shared to encourage more frequent use of PBPK modeling as a quantitative tool to characterize the impact of pharmacokinetics on dose-response assessment, and to reduce uncertainty in regulatory risk assessment for pesticides. *Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.*

1540 The Threshold of Toxicological Concern (TTC) Is a Pragmatic Risk Assessment Tool for the Safety Assessment of Cosmetic Ingredients with Low Consumer Exposure

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The Threshold of Toxicological Concern (TTC) is a pragmatic and conservative tool for risk assessment of substances. The TTC is based on the principle of establishing a human exposure threshold value for all chemicals, below which there is a very low probability of a possible risk to human health. Such threshold values may be identified for many substances on the basis of their chemical structures and the known toxicity of chemicals sharing similar structural characteristics. The TTC concept has been internationally accepted and used in a wide range of regulatory contexts, such as food contact materials, food flavorings, genotoxic impurities in pharmaceuticals and pesticide metabolites in ground water. The TTC approach utilizes human exposure threshold values (TTC values) that have been originally derived from oral toxicity data on cancer and non-cancer toxicity endpoints (Munro et al., 1996). This database has been substantially enlarged by the COSMOS database, an enhanced oral non-cancer TTC dataset on a larger chemical domain, thereby resulting in a new, transparent and public TTC database which also includes 552 cosmetics-related chemicals (Yang et al., 2017). The 5th percentile point of departure (POD) value for each Cramer Class was determined, from which human exposure TTC values have been derived. The COSMOS-plus-Munro federated dataset provided TTC values of 46, 6.2 and 2.3 µg/kg bw/day for Cramer Class I, Cramer Class II and Cramer Class III, respectively. Overall, the TTC is accepted by regulatory authorities and most scientific committees, and there is broad application potential for use in safety assessments of cosmetic ingredients. In order to demonstrate the successful application of the TTC concept not only to cosmetic impurities but also to cosmetic ingredients including hair dyes, fragrances and plant-derived ingredients, Cosmetics Europe has prepared several case studies. Particularly for hair dyes, the comparison with available risk assessments on the basis of *in vivo* data illustrates that the TTC approach is sufficiently conservative and safeguards the safety of the consumer. Overall, we have shown that the TTC concept is not only useful to avoid animal testing but is also successfully used for the safety evaluation of cosmetic ingredients for which human exposures are low or negligible.

1541 *In Vitro-In Vivo* Assay Validation for Vaginal Irritation of Chemicals

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The goal of the study is to validate the utility of the 3D human *in vitro* vaginal tissue model as an alternate for rabbit vaginal irritation using a set of coded test articles (TAs). A double-blinded study was conducted in which the investigator and the animal testing facility were provided with N=14 coded test articles and assayed *in vitro* and *in vivo* following topical application at 2% dose of each TA and 5 repeat exposures over 6 days. Dose volumes were proportionally adjusted based on the surface area and N=5 rabbits and N=3 EpiVaginal tissues were used per TA. While the RVI score was used to monitor *in vivo* irritation; MTT, TEER, and histological analysis were used as endpoints for the *in vitro* assays. The results showed that four TA including two known irritants, benzalkonium chloride (BZK) and nonoxynol 9 (N9) were predicted as irritants by MTT viability and TEER (<50% reduction). While BZK was identified as a mild/severe irritant in the RVI assay, the effect of N9 in rabbits was highly variable - in three independent animal RVI studies, irritation was detected only in 25-30% of the animals. Furthermore, the other two TAs, copper sulfate and sodium dodecyl sulfate (SDS), that were determined as vaginal irritant *in vitro* were not identified as irritant in the RVI test. Additionally, cytokine analysis from culture supernatants showed that N9, SDS and BZK induces a significant increase (>2 fold) in IL-1α/IL-1β release by Epivaginal tissues. In contrast, only BZK showed a significant increase in IL-1α/IL-1β from rabbit vaginal lavages. Based on the *in vitro in vivo* data, we expanded the study to include N=55 test articles and the *in vitro* reproducibility of the results for the 55 test articles were monitored using reconstructed tissues from cells obtained from N=4 donors. In short, a combination of MTT, TEER, and IL-1α/IL-1β were found to be valuable makers of *in vitro* vaginal irritation. In conclusion, the *in vitro* assay method performs equally well or better compared to the standard RVI assay and could be a useful tool to assess vaginal toxicity of formulations, drugs, and medical devices.

1542 Maternal Gestation Engineered Nanomaterial Exposure Reduces Fertility in F1 Females

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We have reported that maternal inhalation exposure to nano-titanium dioxide (nano-TiO_2) during gestation severely reduces circulating estrogen levels and compromises placental efficiency at gestational day (GD) 20 in Sprague Dawley (SD) rats. Alterations to normal gestational milieu neonates are subjected to during development has been extensively shown to significantly contribute to the developmental origins of health and disease. The objective of this study was to determine the effects of gestational exposure on puberty onset, cyclicity, fertility, and pregnancy outcomes. Female, SD rats were housed in the West Virginia University Inhalation Facility under a regulated temperature and 12:12 hour light-dark cycle. Dams of F1 offspring were randomly assigned to either the sham-control or nano-TiO_2 exposure groups and acclimated for 48-72-hours before mating. Inhalation exposures lasted for 6 days after GD 10 to decrease animal stress. The pregnant rats were exposed to an average target concentration of 12 mg/m^3. This exposure paradigm (12 mg/m^3, 6h/exposure, 6 days) produced a calculated lung burden of 525±16 µg. Twenty-four hours after the last exposure dams were sacrificed on GD 20. F1 females (n=3) that were exposed to nano-TiO_2 during development had reduced litter size (3±0.57 pups) when they conceived to the first mating after reaching cyclicity. Additionally, rats born to exposed dams experienced increased time to conception, requiring at least two matings prior to successful conception. Rats that became pregnant to second matings (n=4) had litter sizes (12.5±1.26 pups) comparable to sham-control dams. Dams exposed to nano-TiO_2 also had significantly reduced liter sizes (10.0±0.73) with decreased number of males (4.4±0.38), but not females (6.2±0.88) compared to sham-controls (12.7±0.71, 7.3±1.1, and 5.8±1.1, respectively). Maternal nano-TiO_2 exposure during gestation reduces both liter size and males born compared to sham-control. Furthermore, F1 females born to dams exposed during gestation experienced increased time to conception as well as decreased liter size ultimately leading to decreased fertility. *Support: ES015022 (TRN) U54GM104942 (SH).*

1543 Cytoskeletal and Ultrastructural Alterations Induced by TiO_2 and ZnO Nanoparticles into Antral Follicular Cells

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Titanium dioxide nanoparticles (TiO_2-NP) and zinc oxide nanoparticles (ZnO-NP) are metallic NP widely used in several personal care products, cosmetics, textiles and food. TiO_2-NP have been detected in ovaries from exposed mice, and studies have found that exposure to TiO_2-NP and ZnO-NP alters hormone levels, mating and pregnancy rates. However, no studies have evaluated a direct interaction of NP with ovarian cells. Antral follicles are the functional units of the ovary. They possess steroidogenic activity and are responsible for growing, housing, and promoting competence to the oocyte for fertilization. Previously, we characterized both NP in culture media, and we found impaired antral follicle growth and morphological alterations. This work aimed to evaluate ovarian follicle alterations in the cytoskeleton arrangement and ultrastructure after TiO_2-NP and ZnO-NP exposure. Antral follicles from C57BL6/J mice were cultured with TiO_2-NP (0, 5, 25 and 50 µg/mL) or ZnO-NP (0, 5, 15 and 25 µg/mL) for four days. After culture, immunofluorescence and transmission electron microscopy were performed to determine cytoskeletal and ultrastructural alterations, respectively. Our results show alterations in the cytoskeletal organization in cells from exposed antral follicles, as evidenced by the loss of microtubule boundless irradiating to the periphery and by the appearance of hole-like structures in microtubule such as disorganization in microfilaments. TiO_2-NP were mainly internalized into antral follicles using endocytosis and macropinocytosis transport systems. TiO_2-NP were localized at every follicular cell type but the oocyte. ZnO-NP were not observed in follicular cells because they may dissolve in the culture media. Finally, both NP affected mitochondrial ultrastructure and the trans-zonal projections located from surrounding cumulus cells through the oocyte. Our results suggest that NP elicit toxicity in ovarian follicles via different mechanisms of toxicity, possibly due to their differentiated fate in the culture. *Supported by Fondo de Investigación Científica y Desarrollo Tecnológico del Cinvestav (grant No. 189).*

1544 Subchronic Exposure to DEHP Alters the Expression of Genes Encoding for Proteins Involved in the Extrusion of First Polar Body

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Di (2-ethylhexyl) phthalate (DEHP) is an endocrine disrupting chemical used as a plasticizer that has been related to numerous pathologies including syndromes such as infertility. Previous studies have shown that subchronic exposure to DEHP to environmentally relevant doses impairs the oocyte maturation, evidenced by an increased number of ovulated oocytes that do not reach metaphase II (MII) and do not extrude the first polar body. This study was aimed to evaluate mRNA levels of genes encoding for proteins involved in the extrusion of polar body, including anillin, myosin-9, septin-2 and septin-7. Female CD1 mice were administered orally with 0, 20, 200, or 2000 µg DEHP/kg body weight (bw)/day for 30 days. After the dosing period, females were induced to superovulate by injecting ip 5 IU pregnant mare serum gonadotropin (PMSG), followed by 5 IU human chorionic gonadotropin (hCG) at 48 h later. At 20 h post-hCG injection, oocytes were obtained by washing the oviducts, and then they were classified according to their stage of maturation in germinal vesicular (GV), metaphase I (MI) and MII. Oocytes were subjected to RNA extraction and two-step real time PCR from at least three independent experiments. We found significantly lower mRNA levels of Anillina, myosin-9 and septin-2 at the GV, MI and MII stages of maturation in oocytes from mice treated with 200 and 2000 µg DEHP/kg bw/day compared to controls. Further, we found significantly lower mRNA levels of septin-7 from the MI stage in oocytes from either dose of DEHP compared to controls. These results suggest that DEHP may interfere with genes involved with the extrusion of the first polar body at transcriptional levels for contributing to impair oocyte meiosis.

1545 Levels of Mono-n-butyl Phthalate in the Tissues of Adult CD-1 Female Mice after Repeated Oral Administration of Di-n-butyl Phthalate

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Di-n-butyl phthalate (DBP) is an endocrine disruptor commonly used worldwide as a plasticizer or solvent in many consumer products such as infant care, personal, and cosmetic products. Exposure to phthalates is postulated to cause developmental and reproductive toxicity. While various studies utilizing mice have shown that DBP causes health problems, no quantitative evidence of the accumulation of the active metabolite of DBP, mono-n-butyl phthalate (MBP), in the tissues of exposed female mice currently exists. Previously, we detected MBP in serum (15.74±2.46 µg/ml), liver (2.78±0.43µg/g), and ovary (2.03±0.53 µg/g) of mice treated with a single dose of DBP (1000 mg/kg) after 2 h with the highest concentration of MBP detected 6 h after the DBP dose in serum (19.45 ±5.79 µg/ml), liver (5.22±1.65 µg/g), and ovary (2.77±0.1 µg/g). To investigate the accumulation of MBP after repeated dosing with DBP, we utilized a highly sensitive liquid chromatography/tandem mass spectroscopy assay to determine MBP levels in the liver, serum, and ovary of adult CD-1 female mice after 10 daily oral doses of DBP. We pipet-fed adult mice (n=5 per treatment/time point) with tocopherol-stripped corn oil (vehicle) or ten doses of DBP (1000 mg/kg/day) and collected liver, ovary, and serum at 2, 6, 12, and 24 h after treatment. In mice treated with vehicle, background MBP levels were detected in serum (0.001±0.0002 µg/ml) and liver (0.005±0.012 µg/g), but not in ovaries. Interestingly, there was significant MBP accumulation observed in serum (75.6±10.7 µg/ml, p=0.0014), liver (1255.9±215.3 µg/g, p=0.0013), and ovary (1186.8±201.3 µg/g, p=0.0013) of mice treated with 10 oral dosages of DBP (1000 mg/kg/day) in comparison to the levels detected in mice treated with a single dose of DBP (1000 mg/kg) in our previous study. In the serum, liver, and ovary of mice treated with 10 dosages of DBP the highest concentration of MBP was detected at 2 h after the treatment and decreased with time to undetectable levels at the 24 h time point. In conclusion, we have measured, for the first time, MBP in the tissues of mice after repeated oral exposure to DBP. This study provides evidence that MBP is accumulating in the ovary after oral exposure, thus, it supports a role for direct exposure of this phthalate in the ovary of mice. *This work was supported by NIEHS grant R01026998-01A1 (ZRC).*

1546 The Effects of Oral Dibutyl Phthalate (DBP) Exposure on Transcription Factor Activation in the Mouse Ovary

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Phthalates are a family of endocrine disruptors found ubiquitously in the environment, making the human population susceptible to exposure. Previous research has shown changes in ovarian gene expression following exposures to dibutyl phthalate (DBP). To elucidate the mechanistic effects of DBP, we sought to determine whether oral exposure to DBP alters the activation rate of transcription factors in the ovary. To do so, female CD-1 mice (60 days old, n=14-16 per treatment) were orally administered daily doses of tocopherol-stripped corn oil (vehicle), environmentally relevant doses of DBP (10 and 100 µg/kg/day), or a classical high dose (1000 mg/kg/day) for 10 days. Following dosing, whole ovaries were collected and nuclear protein extracted. Transcription factor activation profiling plate arrays were used to measure the activation of 95 transcription factors in the ovary. A total of 35 transcription factors showed increased activation after DBP exposure, 20 showed decreased activation after DBP exposure, and 40 transcription factors showed differing patterns of increased and decreased activation among the different DBP doses. Our results showed a decrease in NF-E2 activation in the 10 µg/kg/day (p=0.015), 100 µg/kg/day (p=0.05), and 1000 mg/kg/day (p=0.007) groups. Additionally, HOX4C showed increased activation in both the 100 µg/kg/day (p=0.001) and 1000 mg/kg/day (p=0.03) groups. The 1000 mg/kg/day dose also induced an increase in activation for HIF (p=0.05). Lastly, exposure to 100 µg/kg/day induced significant increases in the activation of E2F-1 (p=0.031), COUP-TF (p=0.022), GFI1 (p=0.001), MZF (p=0.007), PAX3 (p=0.011), PAX8 (p=0.018), ROR (p=<0.001), and RXR (p=0.005). These results provide evidence supporting altered transcription factor activation in the ovary following oral exposure to DBP. Interestingly, several of these transcription factors are involved in cell and tissue differentiation and histone modifications. Further studies are necessary to determine the impact of these DBP-induced alterations on these processes. *Supported by NIH R01ES026998 and T32 ES007091-36A1.*

1547 An Environmentally Relevant Phthalate Mixture inhibits Ovulation by Decreasing Prostaglandin Production in Mouse Antral Follicles *In Vitro*

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Phthalates are endocrine-disrupting chemicals that humans are ubiquitously exposed to as a result of their widespread use as plasticizers and solvents in personal care products, building materials, food and beverage containers, and medical supplies. Exposure to these endocrine-disrupting chemicals may impact female reproduction by disrupting the production and metabolism of ovarian prostaglandins, which are integral molecules of the inflammatory response during ovulation. Prostaglandin synthesis is stimulated by the mid-cycle luteinizing hormone (LH) surge that triggers ovulation and formation of the corpus luteum (CL) from the preovulatory antral follicle. This study investigates the hypothesis that phthalates are able to inhibit ovulation by downregulating genes involved in prostaglandin production (*Pla2g4a*, *Ptgs2*, *Ptges*) and transport (*Abcc4*, *Slco2a1*) while increasing prostaglandin metabolic enzymes (*Hpgd*). Isolated antral follicles from CD-1 mice were cultured for 96hr in supplemented media containing follicle-stimulating hormone to induce pre-ovulatory development, and were treated with vehicle control (dimethylsulfoxide, DMSO) or phthalate mixture (PHTmix; 1-500µg/ml). Media were then replaced with maturation media ± hCG (an LH analog) to induce ovulation and treated with DMSO or PHTmix for 0, 4, 11, and 18hr. Next, oocyte release was observed, media were collected for prostaglandin measurements via ELISA, and the follicle/CL was collected for gene expression analysis (n=3-9, p≤0.05). Treatment with PHTmix resulted in a dose-dependent decrease in ovulation at 18hr, with the 500µg/ml dose being statistically equal to the DMSO group that had zero ovulations. Compared to hCG controls, PHTmix decreased *Hpgd* expression and increased *Slco2a1* expression. Further, treatment with PHTmix resulted in dose-dependent decreases in expression of *Ptgs2* with corresponding reductions in the ovulatory mediator prostaglandin E2 when compared to hCG controls. These changes support the hypothesis that PHTmix inhibits ovulation by disrupting prostaglandin production, which may in turn lead to infertility and have negative effects on reproductive health. *Supported by K99/R00ES028748.*

1548 The Effects of a Phthalate Metabolite Mixture on Antral Follicle Growth and Sex Steroid Synthesis in Mice

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Phthalates are used as solvents and plasticizers in a wide variety of consumer products. Most people are exposed to phthalates as parent compounds through ingestion, inhalation, and dermal contact. However, these parent compounds are quickly metabolized to more active compounds in several tissues. Although studies indicate that the phthalate metabolites reach the ovary, little is known about whether they are ovarian toxicants. Thus, this study tested the hypothesis that phthalate metabolites influence the expression of genes involved in sex steroid synthesis, cell cycle regulation, cell death, oxidative stress, and key receptors, as well as production of sex steroid hormones by mouse antral follicles. The selected metabolite mixture consisted of 36.7% monoethyl phthalate, 15.3% monobutyl phthalate, 10.2% monoisobutyl phthalate, 8.2% monobenzyl phthalate, 19.4% mono(2-ethylhexyl) phthalate, and 10.2% monoisononyl phthalate. Antral follicles from adult CD-1 mice were cultured for 96 hours with vehicle control (DMSO) or metabolite mixture (0.065-325 µg/ml). Growth of follicles in culture was monitored every 24 hours. Total RNA was isolated after 24 and 96 hours and used for gene expression analysis. Media were collected and subjected to hormone analysis. Exposure to the phthalate mixture inhibited follicle growth, decreased expression of steroidogenic enzymes, and altered the levels of sex steroids relative to control. The mixture also altered expression of cell cycle regulators, apoptotic factors, and oxidative stress genes, reduced expression of receptors such as *Esr1*, *Esr2*, *Fshr*, *Lhcgr*, and *AhR*, and increased expression of the receptor *Pparg*. Collectively, these data suggest that mixtures of phthalate metabolites can directly impact follicle health.

1549 Phthalate and Phthalate Metabolite Mixtures Alter Gene Expression in Mouse Neonatal Ovaries

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Phthalates are a group of chemicals often used as additives in various consumer products, medical equipment, and personal care products. Although phthalates have short biological half-lives, phthalates and their metabolites are consistently detected in humans, indicating widespread and continuous phthalate exposure. Because a wide range of phthalate structures are used in consumer products, mixtures of phthalates and phthalate metabolites are present in the body. Thus, environmentally relevant mixtures of phthalates and phthalate metabolites were investigated to determine the effects of phthalates on the function of the ovary during the sensitive neonatal period of development. Neonatal ovaries from CD-1 mice were cultured with either DMSO (vehicle control) or phthalate mixture (0.1-100 µg/mL) composed of 35% diethyl phthalate (DEP), 21% di(2-ethylhexyl) phthalate (DEHP), 15% dibutyl phthalate (DBP), 15% diisononyl phthalate (DiNP), 8% diisobutyl phthalate (DiBP), and 5% benzylbutyl phthalate (BBzP). In a second experiment, neonatal ovaries were cultured with either DMSO (vehicle control) or a mixture of the major metabolites of the phthalate mixture (0.1-100 µg/mL) composed of 15% monoethyl phthalate (MEP), 8% mono-(2-ethylhexyl) phthalate (MEHP), 6% monobutyl phthalate (MBP), 4% monoisobutyl phthalate (MiBP), 4% mono-isononyl phthalate (MNP), and 3% mono-benzyl phthalate (MBzP). After 96 hours of culture, ovaries were harvested for gene expression analysis for cell-cycle regulators, apoptosis regulators, and metabolizing enzymes. Phthalate mixture exposure significantly decreased levels of anti-apoptotic factor *Bcl2l10* (1 µg/mL) and cell-cycle regulator *Ccne1* (1 µg/mL), while exposure to the metabolite mixture borderline increased expression of cell proliferation marker *Ki67* (0.1, 1 µg/mL) and pro-apoptotic factor *Casp3* (1 µg/mL). These data suggest that phthalates alter ovarian gene expression and that phthalates and their metabolites differentially impact the developing ovary. *Supported by NIH R01ES028661 and NIH T32ES007326.*

1550 Short-Term Exposure to Di(2-ethylhexyl) Phthalate and Diisononyl Phthalate Disrupts Ovarian Folliculogenesis for as Long as 18 Months following Cessation of Exposure

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Di(2-ethylhexyl) phthalate (DEHP) is a chemical used as a plasticizer in products such as flooring tiles, car upholstery, food containers, and medical equipment. DEHP has become a ubiquitous environmental contaminant because it is used in a variety of products. Studies have shown that DEHP has endocrine disrupting capabilities and can disrupt ovarian folliculogenesis in mice. Diisononyl phthalate (DiNP) is a common DEHP replacement that is rapidly increasing in use and, subsequently, human exposure. Concerningly, some studies have shown that DiNP has negative effects similar to DEHP, but few studies have been done to assess whether DiNP has negative effects on female reproduction. Further, little is known about the consequences of exposure during adulthood to DEHP or DiNP and any persistent effects that may occur after exposure has stopped. Thus, this study tested the hypothesis that adult exposure to DEHP or DiNP negatively affects ovarian folliculogenesis during late-life in female mice. Female CD-1 mice aged 39-40 days were orally dosed with either vehicle control (corn oil), DEHP (20 µg/kg/day-200 mg/kg/day), or DiNP (20 µg/kg/day-200 mg/kg/day) for 10 days. Mice were aged with no additional phthalate exposure and ovaries were collected at 12, 15, and 18 months post-dosing for analysis of ovarian follicle populations. At 12 months post-dosing, DEHP decreased the number of primary and preantral follicles and reduced the total number of follicles in the ovary when compared to control. DiNP decreased primordial follicles and increased preantral and antral follicles when compared to control. At 15 months post-dosing, DEHP and DiNP increased preantral follicles and decreased antral follicles when compared to control. At 18 months post-dosing, DiNP increased primordial follicles and decreased antral follicles when compared to control. Taken together, these data show that short-term exposure to DEHP or DiNP during adulthood affects folliculogenesis more than a year following completion of dosing. *Supported by R56 ES 025147 (JAF), R01 ES 028661 (JAF), Billie A. Field Fellowship (CC), and T32 ES 007326 (CC).*

1551 Prenatal Exposure to Di(2-ethylhexyl) Phthalate and High-Fat Diet Synergistically Disrupts Fetal Oogenesis and Folliculogenesis in Mice

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Di-(2-ethylhexyl) phthalate (DEHP) is a chemical that is widely used as a plasticizer. Exposure to DEHP has been shown to alter ovarian function in humans. Additionally, foods high in fat content, regularly found in the western diet, have been shown to be another potential disruptor of fetal ovarian function. Due to DEHP's lipophilicity, high-fat foods can be easily contaminated. Therefore, exposure to DEHP and high fat diet are both health concerns, especially in pregnant women and the effects of these exposures on fetal oocyte quality and quantity should be elucidated. In this study, our goal was to determine if there are synergistic effects of DEHP exposure at an oogenesis and folliculogenesis. Dams were fed with high fat diet (45 kcal% fat) or a control diet (10 kcal % fat) one week before mating and during pregnancy and lactation. The pregnant mice were dosed with DEHP (20 µg/kg body weight/day) or vehicle control from E 10.5 to litter birth. We discovered that treatment with an environmentally relevant dosage of DEHP and consumption of high-fat diet significantly increases synapsis defects in meiosis and affects folliculogenesis in the F1 generation, indicating DEHP + high-fat consumption can exert adverse effects to several generations.

1552 Investigating Additive Impact of Obesity on Zearalenone-Induced Ovotoxicity

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Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by *Fusarium graminarium* and other *Fusarium* species. Zearalenone and ZEA metabolites bind to estrogen receptors imparting estrogenicity. Additionally, ZEA has negative reproductive female effects, including decreased fertility, changes to external genitalia, reduced litter size, pseudopregnancy, and changes in the estrous cycle. Obesity affects 40% of women and 20% of girls, with higher rates in minority populations in the United States. Obesity impedes female reproduction, causing infertility, poor oocyte quality, miscarriage, and offspring defects. We hypothesized that obesity would enhance ovarian sensitivity to ZEA-induced ovarian toxicity. Female wild type non-agouti KK.Cg-a/a mice (lean) and agouti lethal yellow KK.Cg-Ay/J mice (obese) were fed and given water *ad libitum*. At 6 weeks of age, mice received saline (CT) or ZEA (40 µg/Kg) *per os* for 15 days. Mice were weighed and food intake was quantified twice per week. Daily vaginal cytology was performed every morning to assess the estrous cycle stage. After euthanasia, tissues were collected and weighed. Progesterone and 17ß-Estradiol were measured by ELISA. The abundance of ovarian proteins involved in chemical metabolism (glutathione S-transferase Pi (GSTP1) and microsomal epoxide hydrolase 1 (EPHX1)), DNA damage sensing and repair (ataxia telangiectasia mutated (ATM) and histones H4K16 (acetylated) and H3K9 (trimethylated) and epigenetic modification (histone deacetylase (HDAC) and DNA (cytosine-5)-methyltransferase 3A (DNMT 3A)) were quantified via western blotting. There were no observable effects of ZEA in lean on any of the endpoints examined. In the obese mice, there was no impact ($P > 0.05$) of ZEA on the abundance of ovarian proteins examined, nor did ZEA affect food intake, final body weight, steroid hormone level, or organ weights outside of the reproductive tract. Exposure of obese mice to ZEA decreased ovarian weight ($P < 0.05$), increased uterine weight ($P = 0.08$), increased time spent in estrus ($P < 0.05$) but decreased time spent in the metestrus/diestrus stage of the estrous cycle ($P < 0.05$). Taken together, these data support that obesity is additive to ZEA-induced ovotoxicity and raises concern about the influence of altered physiological status on ovarian xenobiotic exposure effects. *Supported by funding from the Iowa State University Bailey Career Development Award to AFK, the Iowa State University Nutritional Sciences Council Martin Fund to AFK, and the Fulbright Foreign Student Program to EGA.*

1553 Determining Impacts of Glyphosate Exposure on Ovarian Function in Lean and Obese Mice

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Obesity affects approximately 39% of the female population and is associated with increased incidence of reproductive dysfunction and ovarian cancer. Glyphosate (GLY) is a herbicide with high usage in both rural and urban

environments and recent reports have detected both GLY and a major GLY metabolite in human urine. Our previous work discovered an additive impact of obesity on ovotoxicity, thus, we tested the hypothesis that GLY exposure would alter ovarian endpoints and that hyperphagia-induced obesity would be additive to these effects. Female non-agouti KK.Cg-a/a mice (designated lean) and agouti lethal yellow KK.Cg-Ay/J mice (designated obese) were exposed to GLY (2 mg/kg daily *per os*) for 70 days (from 6 to 16 weeks of age). Food intake and body weight were monitored twice weekly, tissues and blood were collected at euthanasia and weighed. Ovarian protein was isolated for the purposes of protein quantification by western blotting. There was no effect of GLY exposure on most endpoints measured in the lean mice, however, GLY exposure reduced ($P < 0.05$) time spent in the metestrus/diestrus stage of the estrous cycle. In the obese mice, GLY exposure increased ($P < 0.05$) feed intake though final body weight was lower ($P < 0.05$). There was a tendency ($P = 0.1$) for decreased time spent in the estrus stage of the estrous cycle in the obese mice exposed to GLY, though no other cyclicity changes were observed. There was no effect of GLY in either the lean or obese mice on estradiol or progesterone levels, organ weights, or on the protein abundance of histone H3 [trimethyl Lys 9] (H3K9me3), ataxia telangiectasia mutated (ATM), or DNA (cytosine-5)-methyltransferase 1 (DNMT1), histone deacetylase 1 (HDAC). Taken together, these data support an effect of GLY exposure on estrous cyclicity in lean mice, but an effect on feed intake, body weight and estrous cyclicity in the obese mice supporting an additive impact of obesity on ovarian effects of GLY exposure. *This work was supported by R21ES026282 and R21ES026282-S1 from the NIEHS.*

1554 Imidacloprid Interferes with Ovarian Antral Follicle Growth, Gene Expression, and Steroidogenesis

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Neonicotinoid pesticides became some of the most popular chemicals in the world following the removal of organophosphorus pesticides from the agricultural market. They are systemic neurotoxicants used to exterminate sucking insects, termites, and fleas. Imidacloprid, the most commonly used member of the neonicotinoid family, is water-soluble and breaks down slowly in the environment, resulting in large-scale environmental accumulation, and chronic exposure of non-target species including humans, birds, fish, and pollinators. Little research has been done to determine whether imidacloprid is an endocrine disrupting chemical (EDC). This study tested the hypothesis that environmentally relevant levels of imidacloprid interfere with antral follicle growth and steroidogenesis. Antral follicles were dissected from ovaries from CD-1 mice (31-41 days old). They were cultured in media treated with dimethyl sulfoxide (DMSO) or imidacloprid (0.2µg/ml, 2µg/ml, 20µg/ml, and 200µg/ml). Follicle growth was measured after 48 hours in culture, and follicles and media were collected separately. The media were used to quantify estradiol, progesterone, and testosterone production via enzyme-linked immunosorbent assays. RNA was extracted from antral follicles and used in qPCR reactions to quantify gene expression of steroidogenic genes. Imidacloprid (0.2µg/ml) inhibited follicle growth, whereas imidacloprid (200µg/ml) increased follicle growth compared to control. Despite having the greatest percent growth, the media collected from the follicles exposed to 200 µg/ml imidacloprid contained the least amount of estradiol compared to control. Imidacloprid at various concentrations altered expression of the aryl hydrocarbon receptor (*AhR*), both estrogen receptors (*Esr1* & *Esr2*), pro-apoptotic and anti-apoptotic factors, cell cycle regulators and steroidogenic enzymes. These data suggest that imidacloprid might follow a nonmonotonic dose response curve, as do many EDCs. Imidacloprid also interferes with cell proliferation, apoptosis, and steroidogenesis. Finally, imidacloprid affects ovarian antral follicles in such a way that growth and estradiol release are not changed in the same direction, and these affects might be mediated via the *Ahr*, *Esr1*, or *Esr2*.

1555 Effect of Nicotine in Diabetic Rat Ovary: A Double-Edged Sword

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Among the components of cigarette smoke, nicotine is known to exert ovarian damage. However, the underlying mechanisms responsible for the toxic effects of nicotine in the diabetic ovary are still to be delineated. Present study was undertaken to evaluate the effect of nicotine in diabetic rat ovary. Female Sprague Dawley rats were randomized into four groups: Group I received citrate buffer and normal saline and served as control, group II received nicotine treatment (100 µg/ml) and served as nicotine control, group III received single dose of streptozotocin (STZ) at the dose of 55 mg/kg and served as diabetic control. Group IV received STZ (55 mg/kg) and nicotine (100 µg/ml) treatment and served as Diabetic + Nicotine. The present results indicated that diabetic animals exhibited more cotinine level as compared to normal nicotine treated animals due to more water intake by diabetic rat. However, nicotine exposure in diabetic rat did not alter the blood glucose level. Results indicated that nicotine *per se* significantly reduced the ovarian weight, further the decrease was observed with Diabetic + Nicotine treated animals, but not statistically significant when compared to *per se* diabetic animals. Results showed that nicotine not only exacerbated the ovarian DNA damage, but also increased the frequency of DNA damage in diabetic ovary. Histological analysis revealed that nicotine further reduced the number of primary follicles in diabetic rat. Furthermore, TUNEL assay results showed that most of the cell death was confined to the ovarian follicles in *per se* diabetic and Diabetic + Nicotine groups. Moreover, to confirm the regulation of ovarian follicular development and steroidogenic capacity of ovarian follicles, 3β-hydroxysteroid dehydrogenase (HSD) immunostaining was performed. Results showed that nicotine treatment led to decrease in the expression of 3β-HSD in the ovary of normal as well as diabetic rat. Further, western blot analysis results showed that nicotine significantly increased the levels of caspase-3, NF-κB and COX-2 expressions in diabetic ovary. Present results showed that nicotine not only exacerbated the DNA damage, but also increased the apoptotic cell death and inflammation in diabetic rat ovary. These findings provide the evidence that nicotine leads to an increase in the inflammatory markers through different signaling pathways and worsens the ovarian reserve in diabetic milieu.

1556 Impact of Heat Stress on Ovarian Chemical Biotransformation Enzyme Abundance

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Heat stress (HS) induces infertility in many production animal species, including swine. During HS, blood is redistributed to the periphery, the intestine becomes "leaky", feed intake is dramatically reduced but paradoxically, animals become hyperinsulinemic. Seasonal infertility occurs due to HS and includes alterations to reproductive endpoints including anovulation and early pregnancy loss. Insulin regulates hepatic and ovarian chemical biotransformation enzymes including microsomal epoxide hydrolase (EPHX1), glutathione S-transferase pi (GSTP1) and cytochrome P450 2E1 (CYP2E1) and our previous work discovered that in another physiological state in which hyperinsulinemia features, obesity, ovarian chemical biotransformation enzymes are altered in abundance and respond differently to ovotoxicants than those of lean females. Thus, the hypothesis for this study is that HS alters abundance of the ovarian enzymes EPHX1, GSTP1 and CYP2E1. Twelve post-pubertal gilts (126.02 ± 21.6 kg) were synchronized for 14 d by orally administering Matrix® to ensure that gilts were heat-stressed during the follicular phase of the estrous cycle. Immediately after Matrix® withdrawal, gilts were split in two groups (n = 6) and were exposed to HS or thermal neutral (TN) conditions for 5 d. TN gilts experienced an average ambient temperature of 20.3°C ± 0.5°C, while the HS gilts was housed in an environment with an average ambient temperature of 31.1°C ± 1.4°C due to cyclical heating to imitate the diurnal pattern. Ovarian protein was isolated and western blotting performed to measure abundance of EPHX1, GSTP1 and CYP2E1. Relative to ovaries from TN gilts, ovarian abundance of EPHX1 was decreased ($P = 0.03$), GSTP1 was unaltered ($P > 0.05$) and CYP2E1 was increased ($P = 0.01$). We have previously noted this opposing abundance pattern between EPHX1 and CYP2E1 during ovotoxicant exposures. These data support that HS affects the capacity of the ovary to metabolize ovotoxicants, potentially contributing to observed infertility. *This work was supported by the Iowa Pork Producers Association (AFK, JWR, LHB).*

1557 Bisphenol S Enhances Gap Junction Intercellular Communication in Human Ovarian Theca Cells

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Gap junctions are intercellular channels composed of two hexameric connexon complexes, allowing the diffusion of molecules <1 kDa between cells. Gap junction intercellular communication (GJIC) occurs through phosphorylation of these connexons. Temporospatial regulation of GJIC is tightly controlled during ovarian follicular development and ovulation. Ovarian steroidogenic theca cells support the structure and vascularity of the developing follicle and need GJIC for steroid hormone trafficking. GJIC can be altered by hormone signaling and exposure to endocrine disrupting chemicals (EDCs), such as bisphenols, EDCs used in the production of polycarbonate plastics. We have previously demonstrated that bisphenol S (BPS), the second most

abundant bisphenol, enhances GJIC in ovine theca cells. Whether this effect is conserved in human theca cells remains unknown. Here, we have investigated if BPS exposure enhances GJIC in human theca cells. To test this hypothesis, ovaries of healthy women (31-48 years old, n=3) without prior history of cancer or hyperandrogenism were collected (IRB: 17-1066M), and theca cells from antral follicles isolated and purified. Cell viability, cytotoxicity, and purity were assessed by trypan blue, MTT assay, and fibulin-5 immunostain, respectively. Cells were grown to ~80% confluency and exposed to control conditions (0.1% DMSO), BPS (200 ng/ml), or a combination of BPS and a panMAPK inhibitor (SB202190) for 24 h. GJIC was assessed using a scrape loading/dye transfer assay. Following BPS exposure in pre-luteinized human theca cells, GJIC was enhanced by >40% compared to the control group. Additionally, we demonstrate that this effect can be blocked by a MAPK inhibitor. This is the first report of BPS-induced GJIC modulation in human ovarian theca cells. Upregulation of GJIC could result in hyperplasia of the theca cell layer, a pathology seen in polycystic ovarian syndrome and hyperandrogenism, or lead to premature ovulation via disrupted steroid signaling. Further studies are necessary to understand 1) if BPS-induced GJIC upregulation is restricted to theca cells and 2) if the BPS-induced GJIC effect seen in theca cells results in altered follicular development and/or poor fertility outcomes. *JG was supported by NICHD T32HD087166. Supported by NIEHS R01ES027863 to A.V-L.*

1558 Vitrification Enables a Long-Term-Storage and Ready-to-Use Murine Ovarian Follicle Bank for High-Throughput Ovotoxicity Screening

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The ovary is the primary female reproductive organ and contains various developmental stages of follicles as the functional unit. The primary functions of follicles are to secrete hormones and to mature germ cell-oocytes for maturation, ovulation, and fertilization. Increasing evidence reveals that a broad spectrum of environmental chemicals and pharmaceutical compounds cause ovarian toxicity (ovotoxicity) and increase women's risks of premature ovarian failure, hormonal imbalance, and infertility. Unfortunately, the lack of optimal *in vitro* models makes the current gold standard for testing the ovotoxicity of chemicals rely on whole laboratory animals. However, *in vivo* models are time consuming, costly, and harmful to animals. In this study, we aimed to use vitrification method to cryopreserve and bank individual mouse ovarian follicles and further integrate our 3D encapsulated *in vitro* follicle growth (eIVFG) model to develop a high-throughput ovotoxicity screening platform. Preantral follicles were isolated from 16-day old CD-1 female mice for vitrification. The vitrified follicles were then warmed and recovered for eIVFG. Results indicated that follicles after vitrification had comparable follicle and oocyte reproductive outcomes with freshly harvested follicles, including follicle survival, follicle development from preantral to antral stage, ovarian steroidogenesis, and oocyte maturation and ovulation. We further used vitrified follicles to screen for the ovotoxicity of doxorubicin (DOX, positive control) and 6 analogs of microcystins (MCs), an emerging category of environmental contaminants from harmful algal blooms (HABs). Our results demonstrated that DOX showed consistent dose-dependent ovotoxicities on vitrified follicles as we previously discovered on freshly harvested follicles, and MCs exhibited endocrine disrupting effects on the ovary. In summary, our study demonstrate that vitrification and eVIFG allow us to establish a long-term-storage and ready-to-use ovarian follicle bank and a high-throughput female reproductive toxicity screening.

1559 Parabens and Phthalates Inhibit the Proliferation of Murine Oviductal Epithelial (MOE) Cells *In Vitro*

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The mammalian oviduct is a central organ for female reproduction as it is the site of fertilization and actively transports the embryo to the uterus. Recent studies, suggest that phthalates and parabens, prevalently found in daily-used products, can impact female reproductive health. Yet, their effects on the oviduct are unknown. Here, we hypothesized that *in vitro* paraben and phthalate exposure of spontaneously immortalized murine oviductal secretory epithelial (MOE) cells would inhibit cell proliferation. To test this hypothesis, MOE cells (400 cells/well) were cultured for 7 days in media containing a wide range of concentrations (0.001-100 µg/mL) of propylparaben, methylparaben, propylparaben + methylparaben, di-2-ethylhexyl phthalate (DEHP), diethyl phthalate (DEP), or DEHP + DEP, or dimethylsulfoxide (DMSO) as vehicle control. Cell proliferation was examined by the sulforhodamine B colorimetric assay, and the optical densitometry readings were used to determine the percent of observed cytotoxicity relative to control. Additionally, we performed a colony formation assay by culturing MOE cells (500 cells/100 mm dish) for 7 days in media containing either DMSO, methylparaben 100 µg/mL, or propylparaben 10 and 100 µg/mL, followed by 4.5 days of culture with only media. Then, the number of colonies was determined with ImageJ. Differences between treatments and DMSO were analyzed by ANOVA and Dunnett post-hoc test (n = 3-5). The results indicate that cell proliferation was reduced compared to DMSO (set as 100%). Specifically, statistically significantly reduction in proliferation ($p \leq 0.05$) were observed at propylparaben 10, 25, 50, and 100 µg/mL (% proliferation ± SD; 33% ± 22, 8% ± 2, 5% ± 3, and 6% ± 4, respectively), methylparaben 25, 50, and 100 µg/mL (70% ± 19, 29% ± 12, and 12% ± 6), propylparaben + methylparaben 5, 12.5, 25, and 50 µg/mL (48% ± 18, 17% ± 9, 6% ± 2, and 3% ± 1), DEHP 10 µg/mL (79% ± 6), DEP 100 µg/mL (74% ± 8), and DEHP + DEP 5, and 50 µg/mL (74% ± 6, and 71% ± 6). Colony formation assays showed statistically significantly decreases in colony numbers ($p \leq 0.05$) between DMSO (mean ± SD; 309 ± 44), methylparaben (223 ± 13), and propylparaben 100 µg/mL (0.3 ± 0.6); whereas propylparaben 10 µg/mL (251 ± 53) was not different from DMSO. Overall, these findings suggest that parabens and phthalates can selectively affect mouse oviductal secretory epithelial cells proliferation and survival, under the examined conditions.

1560 Effect of Bisphenol A on Oxidative Stress, Proliferation, and Differentiation of Human Placental Trophoblast Cells

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Bisphenol A (BPA) is ubiquitous endocrine disruptive chemical suspected to cause adverse effects on human health. BPA has been detected in amniotic fluid, cord blood, placental tissue, and fetal plasma. The presence of BPA in maternal reproductive tissues and fluids is concerning because it suggests that exposure to BPA can occur at all developmental stages and it has potential to affect the reproductive processes in males and females as well as fetal development. Oxidative stress plays a central role in the pathophysiology of miscarriage, pre-eclampsia and fetal growth restriction. Hormones, such as 17β-estradiol (E2), play key roles in the implanting blastocyst and in the process of placentation. In addition, the beta subunit of human chorionic gonadotropin (β-hCG) is secreted by the trophoblast and syncytiotrophoblast and plays a key role in implantation and in the maintenance of pregnancy. Differentiation of cytotrophoblasts (CTBs) into the syncytiotrophoblasts (STBs) is a process required for the development of a functional placenta. This study was aimed to examine the effect of BPA on forskolin-induced cell differentiation and fusion, reactive oxygen species (ROS) production and the release of β-hCG in the human placental trophoblast (BeWo) cells. To induce BeWo cell differentiation, cells were treated with various concentrations of forskolin (FK). Cells were treated with BPA or E2 at various concentrations alone or in combination. Cell proliferation, viability, cell death, ROS production and level of β-hCG were assessed. BPA significantly reduced cell viability in a concentration-dependent manner after 24h of exposure. Low concentrations of BPA significantly potentiates E2-induced cell proliferation. The estrogen antagonist, ICI 182,780, blocked this effect. Treatment of BeWo cells with E2 significantly increased β-hCG. A significant decrease in β-hCG secretion was observed at 0.01nM-10nM BPA. The decreased β-hCG release indicates loss of cell function. Exposure to BPA increased the production of ROS in BeWo cells. Our data suggest that BPA-mediated ROS production can cause oxidative damage and cell death, and any unbalance in β-hCG secretion and steroid hormones action could have serious consequences for pregnancy and fetal development. *Supported by Title III.*

1561 Exploring the Use of Placental Tissue Explants and Trophoblast Cell Lines to Study Toxicity of Anticancer Drugs

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Studying interference with placental function is a useful approach to investigate the safety of drugs during pregnancy, as the placenta is essential in maintaining a healthy pregnancy. Here, we studied the application of placental tissue explants and trophoblast cell lines with respect to assessing the toxic potential of various anticancer drugs. Third trimester placental villous tissue explants (~ 5mg) were cultured for 7 days and exposed for 72h (day 4-7) to a concentration range of doxorubicin, paclitaxel, crizotinib, imatinib or sunitinib. Cell lines representing extravillous trophoblasts (JEG-3), villous trophoblasts (BeWo), and syncytiotrophoblasts (BeWo cells induced with forskolin to syncytialise) were exposed for 48h to the same drugs. Tissue and

cell viability were assessed via a MTT assay and progesterone levels in culture medium were quantified via LCMS/MS. Data are presented as mean ± SEM. We found that placental explants remained viable for 7 days, and all cell lines for 48h. In placental explants, progesterone release ranged from 0.01 to 3.3 ng·mg^{-1} wet weight ·24h^{-1} under control conditions. In syncytialised BeWo cells, progesterone production was on average 174 ± 11 ng·mg^{-1} protein ·48h^{-1} under control conditions and 2.12 ± 0.12 fold higher than in BeWo cells. Paclitaxel did not affect explant viability, whereas it reduced cell viability to 50% or less in all cell lines at 3 - 10 nM. Furthermore, it was consistently less cytotoxic in syncytialised BeWo cells compared to BeWo and JEG-3 cells. Doxorubicin (1 μM) reduced viability in explants to 83 ± 7%, whereas it fully inhibited viability in all cell types. For crizotinib, sunitinib and imatinib differences between cells and explants were less profound. Interference with progesterone release in explants could not be studied due to a large variability in measurements, but syncytialised BeWo cells proved suitable for this purpose. We found that 1 μM sunitinib significantly reduced progesterone release to 76 ± 6%, without affecting cell viability. For the other compounds no major effects on endocrine function were seen. In conclusion, placental explants are a suitable model for testing effects of drugs on tissue viability. The additional use of cell models may provide information on the type of trophoblast that is most prone to cytotoxicity. Furthermore, syncytialised BeWo cells proved suitable for studying interference with steroidogenesis.

PS 1562 17-Alpha Hydroxyprogesterone Caproate (17p) Alters the Nitric Oxide Signaling Pathway in an *In Vitro* Placental Cell Culture System

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Preterm birth (PTB) impacts 1 in 10 pregnancies annually in the United States. A current therapy for recurrent spontaneous PTB prevention is 17-alpha hydroxyprogesterone caproate (17P), a synthetic progestogen. While the precise mechanism of action is not well established, the therapeutic effects of 17P may be driven by an inhibition of nitric oxide (NO) signaling. To understand the mechanism of 17P, gene and protein expression experiments were conducted. Initially, a cytotoxicity assay was performed to determine the appropriate doses of 17P. JEG-3 trophoblasts were treated with 17P for 24h at doses ranging from 0.1 to 100ug/mL. RT-PCR was conducted to detect changes in mRNA expression of seven inflammation-related genes such as endothelial Nitric Oxide Synthase (*eNOS*), Vascular Endothelial Growth Factor (*VEGF*), interleukin-8 (*IL8*), Chemokine Ligand 2 (*CCL2*), matrix metalloprotein-1 (*MMP1*), and other target genes of the NO signaling pathway. In addition, protein expression was assessed using Western Blot. Cytotoxicity was observed at or above doses 30ug/mL. Based on these results, doses of 0.3-10ug/mL were selected for gene and protein expression studies. Cells treated with 17P showed a significant dose-dependent increase in *VEGF* and *eNOS*. A slight increase in IL6 and MMP1 gene expression and dose-dependent decreases in IL4 and CCL2 gene expression were noted after exposure to 17P. Dose-dependent decreases were observed in IL4 and *CCL2*. Under the same treatment conditions, JEG-3 cells were also assessed for intra- and extra-cellular NO content using a modified Griess reagent assay. These data provide evidence of a NO-dependent mechanism of action of 17P.

PS 1563 Development of Fibrosis in Endometriotic Cells Is NR4A1 Dependent and Can Be Inhibited by NR4A1 Antagonists

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Endometriosis is an inflammatory and highly painful disease that is treated by hormonal therapies and this can be problematic for women of reproductive age. Thus there is urgent need for development of alternative therapies. We recently reported that NR4A1 promoted growth and activated mTOR signaling in Ishikawa endometrial cancer cells (a model for epithelial-derived endometriosis) (1) and found similar results in stromal-derived endometriotic cells from patients. Some of the currently used therapies are toxic, whereas the NR4A1 ligands (C-DIMs) are relatively non-toxic. In this study, we showed that knockdown of NR4A1 by RNA interference in epithelial or stromal-derived endometriotic cells thus inhibited fibrosis and this was accompanied by decrease α-smooth muscle actin (α-SMA) and related fibrotic genes including collagen Type I alpha1 (COL1A1), connective tissue growth factor (CTGF) and Fibronectin (FN). These results demonstrate the pro-fibrotic activity of NR4A1 in endometriosis and this was in contrast to a report from another laboratory which suggested the NR4A1 repressed fibrosis. We also treated endometriotic cells with two different bis-indole derived compounds which act as NR4A1 antagonists, namely, 1,1-bis (3′-indolyl)-1-(p-hydroxyphenyl)methane (CDIM8, DIM-C-pPhOH) and a second generation buttressed analog of CDIM8, 1,1-bis (3′-indolyl)-1-(3-chloro-4-hydroxy -5-methoxy)methane (DIM-C-pPhOH-3-Cl-5-OCH_3) which act as NR4A1 antagonists. Treatment of endometriotic cells with DIM-C-pPhOH or DIM-C-pPhOH-3-Cl-5-OCH_3 gave results comparable to those observed after NR4A1 knockdown, namely there was a decrease in expression of α-SMA, COL1A1, CTGF and FN. Moreover, the buttressed DIM-C-pPhOH-3-Cl-5-OCH_3 analog was more potent than the parent compound. In summary, our results confirm that NR4A1 represents a druggable target for treating endometriosis with bis-indole derived NR4A1 antagonists. *References: 1. K Mohankumar, X Li, S Sridharan, K Karki, S Safe. Nuclear receptor 4A1 (NR4A1) antagonists induce ROS-dependent inhibition of mTOR signaling in endometrial cancer. Gynecol Oncol. 2019; 154(1):218-227.*

PS 1564 Maternal Health and Placental Outcomes after Pulmonary Nanoplastic Exposure

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Accumulating plastic waste in the environment has recently led to increasing concerns pertaining to human health. Microplastic concentrations have been measured in the environment, bottled water, food, and human feces. Studies have also demonstrated the ability of plastic to fragment into smaller particles, producing nanoplastics. Though methods of nanoplastic detection and quantification are still in development, the breakdown of micro- to nano-sized particles has been identified. Our lab has shown nanoparticles produced from metallic bulk material can cause adverse pregnancy outcomes after maternal inhalation, including fetal growth restriction. However, maternal-fetal outcomes and placental toxicity after nanoplastic exposure remain unexplored. Here we sought to begin the evaluation of gestational health after pulmonary nanopolystyrene exposure. Sprague-Dawley rats were administered 300 μL of either 20 nm rhodamine-labeled polystyrene (PS) beads (8.8 x 10^{14} particles/mL) or saline on gestational day 19. Animals were sacrificed 24 hours later and tissues were collected for assessment. Dams who were administered PS had significantly more circulating 17β- estradiol (1104.5 ± 68.1 pg/mL vs 972.0 ± 18.4 pg/mL) and hCGβ (53.4 ± 12.0 pg/mL vs. 6.3 ± 7.5 pg/mL) compared to saline controls. Placental weights in the exposed group were also significantly increased (0.522 ± 0.06 g vs 0.472 ± 0.08 g), with no compensatory increase in fetal weight (2.6 ± 0.7 vs 2.6 ± 0.1). Therefore assessment of placental efficiency, identified as the ratio of placental to fetal mass, was decreased (4.8 ± 0.53 vs 5.5 ± 0.74) in the exposed group. Overall, these studies demonstrate a disruption to maternal endocrine homeostasis during late-stage pregnancy after PS pulmonary exposure. Further, placental mass is elevated within 24 hours of a single maternal pulmonary exposure, which may be due to PS translocation and/or placental inflammation. Increases in hCG production and placental weight without increase in fetal weight indicate placental adaptation to nanoplastic exposure, but with negative effect on placental efficiency. If sustained, these findings suggest maternal compensation to maintain fetal growth and development after PS exposure. *Supported by: NIH-R00-ES024783 (PAS); T32-ES007148 (JND); P30-ES005022.*

PS 1565 Cigarette Smoke Condensate Exposure Activates Aryl Hydrocarbon Receptor Signaling in Human Trophoblast Stem Cells and Alters Markers of Growth and Differentiation

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Although the association of cigarette smoke with increased risk of poor birth outcomes such as low birth weight and neonatal mortality is well-established, we do not understand the exact mechanism of these outcomes. Cigarette smoke contains thousands of chemicals known or suspected to cause disease. The placenta is an essential organ for fetal development. Hemochorial placentation is characterized by the differentiation of trophoblast cells specialized to interact with uterine and fetal vascular beds. Human trophoblast stem (TS) cells provide an *in vitro* model for testing the effects of environmental toxins such as cigarette smoke condensate (CSC) on differentiation and function. In this study, we utilized RNA-Seq analysis to examine changes in gene expression in human TS cells following exposure to CSC. About 900 transcripts were significantly (padj < 0.05) altered. *CYP1A1* and *CYP1B1* were among the top induced transcripts. The top affected associated network includes genes mapping to cellular assembly and organization, DNA replication, repair, and cellular development. Transcripts that were significantly downregulated by CSC include *TAGLN*, a marker of smooth muscle differentiation; *ACTA1* which plays a role in cell motility, structure and integrity; *HAND1*, a key transcription factor for trophoblast cell differentiation; and *ASCL2* which is crucial for the

differentiation of progenitor cell populations into specialized trophoblast cell types. Significant increases were observed for transcripts such as *AMER2*, a negative regulator of canonical Wnt signaling; *ID2*, which antagonizes the activities of basic helix-loop-helix transcription factors; and *GCM1*, involved in the control of placenta-specific gene expression. These data suggest that gene expression in human TS cells is significantly altered by cigarette smoke exposure including via the AhR pathway and genes encoding proteins implicated in the regulation of trophoblast cell differentiation and function. These alterations may impair proper development and function of the placenta and pregnancy outcomes. Ongoing studies are assessing the specific role of AhR signaling in trophoblast differentiation and function. *Supported by the Cross Family Foundation CPGM Pilot Award and NIH R01ES029280.*

1566 Regulation of Mouse Uterine Aquaporin Expression in the Preimplantation Period and the Impact of Ovarian Superovulation

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Uterine fluid level in early pregnancy is a critical determinant of fertility and aquaporins (AQPs), selective bidirectional transporters that move water along a concentration gradient, are major regulators of water homeostasis. The expression of mouse uterine AQP genes and proteins in early pregnancy has been studied but these studies yielded inconsistent results. To better understand their roles in regulating luminal fluid levels in the preimplantation period, we conducted a multi-pronged analysis of uterine *Aqp*/AQP expression on the morning of D1 and D4 of pregnancy in ovarian superovulated and non-superovulated WT C57 Bl/6 mice. Expression was also determined in ovariectomized mice that received E2, P4 or E2 + P4 and in D4 pregnant RU486-treated mice. Our analyses revealed that in the non-superovulated females, *Aqp3*, *Aqp4*, *Aqp5* and *Aqp8* exhibit greater expression on D1 and are induced by E2 while *Aqp1* and *Aqp11* exhibit greater expression on D4 and are induced by P4. Interestingly, *Aqp9* is constitutively expressed. We also observed that P4 inhibits the E2-induced expression of *Aqp3* and *Aqp4* (with the negative effect on *Aqp3* being transient) while *Aqp1* and *Aqp11* are negatively regulated by E2. Superovulation increased *Aqp1*, *Aqp3*, *Aqp5* and *Aqp8* expression on D4 and triggered dramatic spatial changes in AQP expression in both D1 and D4 uteri. These aberrant changes in expression following superovulation likely reduce implantation success and might be managed by targeting E2 or P4 levels and actions in the uterus during *in vitro* fertilization and embryo transfer (IVF/ET).

1567 Glyphosate's Role in the Male Fertility Epidemic and Its Effect on Spermatogenesis

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Since being introduced in 1974, 1.8 million tons of Glyphosate have been sprayed on crops in the US, thus representing the #1 herbicide used in the United States. Recently, studies have shown that glyphosate is capable of acting as an endocrine disruptor and thus may impact male fertility. In the last 40 years, semen parameters in Western men have declined by as much as 50%. Exposure to ubiquitous environmental toxicants could explain this sharp decline in semen parameters. To investigate whether glyphosate directly impacts the male germline, we utilized our novel *in vitro* spermatogenesis model to determine if glyphosate affects differentiation, proliferation, and viability of spermatogenic cells. This study shows that at residential and occupational use levels, glyphosate exposure increased the percentage of apoptosis in male germline cells. We hypothesize that glyphosate may induce cell death in *in vitro* spermatogenic cells through receptor mediated apoptosis pathways such as TRAIL, TNFa, FasL, NADD, and FADD.

1568 Establishment of a Historical Database for Normal Cynomolgus Macaque Spermatozoa Evaluation in Male Reproductive Toxicology Studies

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ICH guidance S5(R2) recommends the assessment of sperm count, motility, and morphology to help confirm or characterize effects of pharmaceuticals on male reproduction, with the cynomolgus macaque commonly used as a test system. However, S5(R2) highlights that non-human primates lack historical background data and may differ kinetically from humans and that animal numbers used are too low for detection of risk. Additionally, studies using cynomolgus macaques are typically analyzed via the WHO Laboratory Manual for the Examination and Processing of Human Semen (currently Fifth Ed., 2010), as few publications exist specifically describing the normal cynomolgus macaque sperm. To assist and improve the assessment of cynomolgus macaque sperm for toxicology studies, historical sperm counts/concentration, motility, and morphology assessments were collated from over 400 non-human primates between 2007 and 2019. All animals were either facility stock or had been dosed with a control/sham article. Sperm was collected via direct penile electrostimulation, with ejaculate volume recorded and motility and sperm count assessed using the TOX IVOS or TOX IVOS II computerized assisted sperm analysis (CASA) systems. Morphology was evaluated using light microscopy. Potential differences due to age and origin were also evaluated.

1569 Estrogen-Related Receptor Is Critical Player in Di-butyl Phthalate-Mediated Male Reproductive Toxicity

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Growing incidences of male infertility in the past few years in part due to exposure to environmental chemicals is a global concern. These xenobiotics disrupt endocrine function and/or cause oxidative stress adversely affecting male fertility. However, knowledge of the molecular mechanisms and the associated candidates underlying chemical mediated male sub-fertility is limited. Di-butyl phthalate (DBP), commonly used as plasticizer in food and water containers, is known to affect male fertility. Therefore, an attempt was made to identify the mechanisms/candidates underlying DBP mediated reduction in male fertility using *Drosophila melanogaster*, an already established model for male reproductive toxicity studies. We observed that DBP exposed males had reduced fertility like in mammals. These males had mislocalized as well as reduced mitochondria with altered membrane potential in testes. Further, ultrastructural analysis involving the apical region of testes in DBP exposed males revealed round swollen mitochondria with deformed cristae. Also, transcripts/proteins associated with mitochondrial biogenesis (cdk4, NRF1, NRF2, cyclin D), mitochondrial dynamics (drp1, mitofusin) as well as mitophagy (dpink and parkin) were misregulated in testes/sperm from DBP exposed males. Besides, ATP-5α Synthase (marker of ATP synthesis) levels were significantly reduced in testes/sperm suggesting functional alterations also in mitochondria from DBP exposed males. Mutational/genetic analyses revealed that these phenotypes parallel those observed in the *Drosophila* testes lacking Estrogen-Related Receptor (dERR), a steroid hormone receptor known to act in carbohydrate metabolism and mitochondrial homeostasis. Interestingly, the transcript and activity levels of dERR were altered by DBP exposure Consistent with this, DBP exposed males had metabolic hallmarks typical of ERR knockdown and testes specific depletion of dERR attenuated DBP mediated reduction in male fertility. Therefore, estrogen related receptor is critical for DBP mediated male reproductive toxicity. To conclude, DBP mediated modulation of ERR might generate non-functional defective mitochondria thereby affecting the testicular/sperm energetics leading to reduced male fertility.

1570 Evidence of Oxidative Damage and Reproductive Dysfunction Accompanying Di-n-butyl Phthalate Exposure in Male Wistar Rats

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Di-n-butyl phthalate (DnBP) is a widely reported chemical used in manufacturing household products, rocket fuel and paints, has been noted to pose lot of danger to the body due to its toxicity, causing a great health challenge and concern. This study aimed at providing evidence regarding the DBP-induced reproductive dysfunction in male Wistar rats. Twenty-eight Male Wistar was randomly divided into four groups (n=7). The rats were orally administered with vehicle, DBP (100, 250 and 500 mg/kg) for twenty-eight days. Thereafter, sperm kinematics, sperm motility, selected biomarkers of oxidative damage, endocrine disruption, inflammation, and DNA damage was evaluated. DnBP-treated rats showed decrease in the body weight gain, testis and epididymis weights. The cauda epididymal sperm total motility, progressive motility and epididymal sperm concentration, curvilinear velocity, straight line velocity, average path velocity, linearity, straightness, beat/cross-frequency were significantly decreased ($p<0.05$) in all treated groups. Increased levels of sperm abnormality, non-progressive motility, wobble and immotility was observed in all treated groups. DnBP increased testicular and epididymal malondialdehyde (MDA) level and hydrogen peroxide generation, but significantly decreases ($p<0.05$) Catalase (CAT), Superoxide dismutase (SOD), Glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione S-transferase (GST) activities in the testes and epididymis. Also, DnBP induced hormonal imbalance, markedly increase expressions of testicular cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and 8-hydroxy2-deoxyguanosine (8-OHdG). Histological study revealed moderate congestion of interstitial vessels, oedema in the testes, fibrotic connective tissues and lack spermatozoa storage in the epididymis of DnBP treated rats. Overall, DnBP induced reproductive dysfunctions as well as inflammation and DNA damage in male rats.

1571 Phthalate-Induced Testicular Injury Results in an Increase of Peritubular Macrophages and Consequent Increase in Differentiating Spermatogonia

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Peripubertal exposure to the phthalate metabolite mono-(2-ethylhexyl) phthalate (MEHP) in rodents causes testicular inflammation, spermatocyte apoptosis, and disruption of the blood-testis barrier. The MEHP-induced inflammation response includes an infiltration of macrophages and neutrophils to the testes, although the cause and purpose of this response is unknown. Recently, a population of testicular macrophages phenotypically distinct from those which are resident in the interstitium was described in mice. Testicular peritubular macrophages aggregate near the spermatogonial stem cell niche and are believed to stimulate their differentiation. We hypothesize that if testicular peritubular macrophages do indeed stimulate spermatogonial differentiation, MEHP exposure would result in an increase of peritubular macrophages to stimulate the replacement of lost spermatocytes. Male rats were exposed to 700 mg/kg MEHP or corn oil (vehicle control) via oral gavage at PND 28 and euthanized at 48 hours, 1 week, or 2 weeks later. Tubules were stained with immunofluorescent markers for macrophages and undifferentiated spermatogonia. Peritubular macrophages were observed in rat testis similar to those previously described in mice: MHC-II+ cells on the surface of seminiferous tubules with heterogeneous morphology. Quantification of MHC-II+ cells revealed that, unlike in the mouse, their numbers did not increase through puberty. MEHP increased macrophage presence by six-fold 48-hours after exposure and remained elevated by two-fold two weeks after exposure. An increase of differentiating spermatogonia occurred two weeks after MEHP exposure. Taken together, our results suggest that peritubular macrophages play a crucial role in the testis response to acute injury and the subsequent recovery of spermatogenesis.

1572 Dipentyl Phthalate Induces Multinucleated Germ Cells and Reduces Testosterone in the Rat Fetal Testis with a Similar Dose-Response

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Phthalic acid esters (phthalates) are male reproductive toxicants that exert their most potent toxicity during a window of sensitivity in fetal development. In the fetal rat, exposure to phthalates reduces testosterone biosynthesis, alters the development of seminiferous cords, and induces the formation of abnormal multinucleated germ cells (MNGs). While human and mouse fetal testes may be less sensitive to the anti-androgenic effects of phthalates than the rat, our previous work demonstrated that phthalates with medium-length side chains cause both induction of MNGs and reduction of testosterone in the rat fetal testis. In this experiment, we sought to compare the relative sensitivity of testosterone reduction and MNG induction as markers of phthalate toxicity, using dipentyl phthalate (DPeP), a known toxic phthalate, and mono-(2-ethylhexyl) tetrabromophthalate (TBPH), a suspected toxic phthalate. We also sought to develop an improved image analysis method for identification of MNGs in histological sections using a neural network approach. Timed pregnant Sprague Dawley rats were exposed to 1-100 mg/kg/d DPeP, 62.5-500 mg/kg/d TBPH, or corn oil vehicle, by daily oral gavage from gestation day (GD) 16-20. Fetal testes were isolated on GD 20 for analysis of testosterone production and quantification of MNGs in histological sections. We found that DPeP induced MNGs and decreased testosterone production with similar dose response. TBPH, conversely, did not exert any toxic effect at any dose up to 500 mg/kg/d. For image analysis, we trained a convolutional neural network with a U-Net architecture using hand-labeled images with human-identified MNGs, to identify MNGs on unlabeled images. With hand-labeled images not used in model training, we assessed the performance of the model, finding it comparable to a human. We conclude that induction of MNGs is a dose-dependent marker of DPeP exposure, with sensitivity similar to testosterone production, which may be generalizable to other phthalates. The use of automated image analysis will allow data on this histopathological endpoint to be more readily collected for analysis of phthalate toxicity. *Abstract does not necessarily reflect US EPA policy.*

1573 Mono-(2-ethylhexyl) Phthalate Enhances All-Trans Retinoic Acid Toxicity in *Ex Vivo* Cultured Fetal Mouse Testis

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Phthalates are a class of chemicals that cause developmental and reproductive toxicity. Phthalates are used primarily as plasticizers in polyvinyl chloride plastics and are found in medical devices, industrial, and commercial products. Human exposure to phthalates is pervasive because they are not covalently bound to plastic polymers. Despite their known adverse effects on male reproductive development, there are significant gaps in the current understanding of the mechanisms of phthalate toxicity. Retinoic acid signaling is involved in both spermatogenesis and fetal gonad development. There is evidence that phthalates disrupt retinoic acid signaling, which could contribute to phthalate toxicity. However, the contribution of this interaction to phthalate toxicity is unclear. In this study, we hypothesized that mono-(2-ethylhexyl) phthalate (MEHP) would enhance the toxicity of all-trans retinoic acid (ATRA) during mouse fetal testis development. To test this hypothesis, gestation day 14 C57BL/6 mouse testes were isolated and cultured on media containing MEHP (10^{-4}, 10^{-5}, or 10^{-6} M), ATRA (10^{-6}, 10^{-7}, or 10^{-8} M), a co-exposure consisting of 10^{-6} M ATRA with the full concentration range of MEHP, or vehicle control (1:4000 DMSO). After 3-day culture, tissues were fixed for histological analysis including quantification of germ cells and seminiferous cords per testis section. Immunofluorescence was performed to assess the number of germ cells, labeled with GCNA, induction of meiosis, indicated by STRA8, SOX9-positive Sertoli cell number, and abnormal FOXL2-positive somatic cells. Exposure to ATRA disrupted seminiferous cord morphogenesis, stimulated the expression of the ovarian protein, FOXL2, and enhanced germ cell meiotic entry. However, the number of SOX9-positive cells was not altered in a dose-dependent fashion. Germ cell count was slightly reduced in the ATRA and co-exposure treatments, but this was not significant. Co-exposure to MEHP and ATRA increased the number of FOXL2-positive cells and reduced seminiferous cord number, relative to ATRA alone. However, the co-exposure did not modify the impact of ATRA on STRA8 expression. We conclude that MEHP enhances some adverse effects of ATRA in the fetal mouse testis, suggesting an interaction between MEHP and the retinoic acid signaling pathway.

1574 Flusilazole Affects Retinoic Acid Signaling in Fetal Rodent Testes

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Azoles are widely used anti-fungal agents used for both medicine and crop protection. In fungi they typically work by damaging the cell membrane by inhibition of cytochrome P450 enzymes, primarily CYP51. However, azoles can have endocrine disrupting effects in mammals and fetal exposure to certain azoles can disrupt reproductive development. We recently showed that the agricultural fungicide flusilazole has potent anti-androgenic effects *in vitro*, but less so *in vivo*. Rats exposed *in utero* did not display classic anti-androgenic effects such as shorter anogenital distance. However, the sex hormone profile was disrupted in male fetuses, with higher androstenedione and lower estrogen levels. This suggests reduced CYP19 (aromatase) activity. Since the *in vitro* effects did not predict the *in vivo* effects, however, we aimed to characterize additional mechanisms for which azoles potentially can disrupt reproductive development. Using *ex vivo* rat gonad cultures we found that flusilazole also affects retinoic acid signalling in mammals, for instance disrupted *Dax1* expression; a key gene for mammalian gonad differentiation. In this project, we are exploring further, using explanted gonads from transgenic mouse strains, how flusilazole - and environmental chemicals more broadly - can disrupt gonadal sex differentiation, which can have severe consequences for reproductive development and function.

1575 Comprehensive Protein Expression Analysis in Rat Testis at Repeated Administration of Testicular Toxicants

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Some chemicals have testicular toxicity to animals and humans: ethylene glycol monomethyl ether (EGME) and 1,3-dinitrobenzene (1,3-DNB), etc. However, their primary toxic targets are different each other: spermatocytes and Sertoli cells, etc, and their detailed mechanisms of toxicity are unclear. In addition, comprehensive protein expression analysis (proteomics) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) is useful for understanding biological reactions in the tissue, and its use has been increasing in recent years. Therefore, in this study, we analyzed the changes in protein expression during repeated administration of testicular toxicants with different toxic targets, and estimated their respective toxic mechanisms and related biological responses. Test substances were administrated once a day to 8-week-old male SD rats for up to 14 days, and the testes collected from rats were subjected to organ weight measurement, histopathological examination, and proteomics by LC-MS/MS. Pathway analysis were performed for these expression data by Ingenuity Pathway Analysis (IPA) (QIAGEN Bioinformatics). Histopathological examination observed the morphological changes characteristic of each test substance: degeneration of spermatocytes by EGME, and vacuolation in seminiferous tubules by 1,3-DNB, etc. As a result of comprehensive protein expression analysis, changes were observed in various pathways: enzyme induction, redox reaction, energy production, etc. Some pathways showed common changes to test substances, and the other showed different patterns for each test substance. In this presentation, based on these results, we will discuss and report the relationship between toxic target differences and patterns of pathway changing.

1576 Characterization of Rat Testicular Organoids Exposed to Ionizing Radiation

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Exposure of male germ cells or somatic testicular cells to certain chemical or physical agents can result in testicular toxicities that adversely affect fertility or offspring. However, suitable *in vitro* models for investigating toxic events in testicular cells are lacking. Therefore, we developed an *in vitro* testicular organoid model using cells from postnatal day 20 (PND20) rat testes. Immunolabelling (IL) was used to characterize the cell types and cellular architecture within the testicular organoids. The testicular organoids were exposed to 0, 2, and 5 Gray (Gy) ionizing radiation (IR) and the media was collected at 0, 2, and 4 hours post-IR. Lactate and lactate dehydrogenase (LDH) levels were measured using the media to assess metabolic function and cytotoxicity, respectively. At 4 hours post-irradiation, mRNA was isolated from the organoids and used to quantify IR-induced gene expression levels. IL analysis revealed that the organoids contain seminiferous tubule-like structures (STSs) with a Sertoli cell barrier (SCB). Spermatogonial stem cells (SSCs) and undifferentiated spermatogonia were also associated with the STSs. IR exposure decreased lactate and increased LDH release in a dose-dependent manner. A ≥ 2-fold change in expression was observed in several genes involved in toxicity and male fertility. These data reveal that the testicular organoids possess cell types and cellular architecture resembling the *in vivo* testes and are capable of producing detectable changes in metabolic function, cytotoxicity, and gene expression following IR treatment. These preliminary results suggest that rat testicular organoids could be a suitable *in vitro* model for evaluating testicular toxicity.

1577 Small RNAs in Rat Sperm as Biomarker of Exposure to Testicular Toxicant Methotrexate

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Methotrexate (MTX), a widely prescribed drug to treat neoplastic, autoimmune and inflammatory diseases, can lead to the inhibition of DNA and RNA synthesis, causing cytotoxicity and genotoxicity. In particular, testicular injury has been observed with MTX in both preclinical models and clinically. The present study established a low-dose subchronic MTX exposure model in the rat with the ultimate goal of identifying distinct sperm RNA expression patterns associated with testicular injury and creating novel biomarker panels for testicular toxicity assessment. MTX was administrated intraperitoneally (IP) at the dose of 2, 5 and 10mg/kg to adult male Fisher rats (n=10) weekly for 13 weeks. The animals were sacrificed one week after the last MTX injection. To characterize the testicular injuries, testes/epididymal weights, sperm count, sperm motility and testes/epididymal histology were assessed. Moreover, total RNA was isolated from epididymal sperms and small RNA was profiled using next generation RNA-sequencing. Sperm count and motility were similar among the control and treated groups. MTX treatment at 10mg/kg significantly decreased testes weight. There was a clear dose-related effect of MTX exposure on testicular histopathology, including a loss of spermatocytes in late stages of the seminiferous epithelial cycle and a loss of round spermatids in early stages of the cycle, resulting in seminiferous epithelial thinning and decreased seminiferous tubule diameter. Neither retained spermatid heads (RSH) nor Sertoli cell vacuoles were a prominent feature, only being observed in a minority of the high dose rats. Significant dose-dependent changes in piRNA length and piRNA length-distributions were identified from sequencing analysis. These data showed that sub-chronical low-dose MTX cause testicular histopathological abnormalities, and sperm piRNAs can be developed as useful biomarkers of MTX-induced testicular injury.

1578 An mTOR-Dependent Regulation of the Blood-Testis Barrier: A Potential Novel Mechanism of Male Reproductive Toxicity

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Alarming decline in sperm quality in terms of number and motility observed in recent years is a clear indication of malfunctioning of male reproductive system. Environmental toxicants such as PBDEs demonstrate strong relationship with defected spermatogenesis. PBDEs have been also shown to modulate mTOR pathway. It was demonstrated recently that activity of mTORC1 promotes opening of the Blood Testes Barrier (BTB) and activity of mTORC2 promotes its integrity. Thus, we hypothesize that one possible pathway through which environmental toxicants, e.g. PBDE, may exert reproductive toxicity consists in the mTOR-dependent modulation of the BTB permeability. Sertoli cell specific knockout mice for Raptor (mTORC1) & Rictor (mTORC2) were generated by breeding animals with floxed target genes and transgenic animals with Cre controlled by Amh promoter. Six animals from each group were sacrificed at postnatal weeks 8, 12, 22 and one year of age to analyze changes in testes weight, sperm count & morphology followed by biotin tracer assay to assess the BTB integrity. Sperm DNA was used to measure mitochondrial copy number and telomere length. Significant reduction in testes weight and sperm count were observed in both knockout types as compared to wild type. The mTORC2 knockouts showed an age-related increase in abnormal spermatozoa, mitochondrial copy and telomere length as compared to the mTORC1 knockout and WT. Biotin tracer assay reveled intact BTB in mTORC1 knockouts and wild type animals while the mTORC2 knockouts showed compromised BTB. Moreover, the histology of testes showed a degradation of seminiferous epithelium in mTORC2 knockouts. Together these data suggest that chemical compounds that affect balance of mTOR complexes may exert reproductive toxicity via altered permeability of the BTB.

1579 Amino Acid/Lipid Profiling in Plasma of Rats with Chronic Inflammation Induced by Endocrine-Disrupting Chemicals and Sex Hormone

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Estrogen and testosterone regulate prostate growth, and endocrine disrupting chemicals (EDCs) can lead to prostate disease development. Elevated estrogen levels can occur during aging and/or reduced testosterone levels. Thus, cases of prostatitis in young men are most likely to have unknown causes. Blood levels of lipids and amino acids have been investigated as potential biomarkers for many diseases, including prostate cancer, yet there are few studies to examine lipid and amino acids from plasma samples from prostatitis patients. Our previous study found that chronic inflammation in the prostates of Sprague-Dawley (Hsd:SD) rats was induced by estradiol benzoate (EB; EDC), and testosterone (T)/estradiol (E). Pups on postnatal days (PNDs) 1, 3, and 5 were injected with EB, and then additional E and T exposure were performed via silastic tube implants in the subcutaneous region from PND 90 through PND 200. To determine whether chronic inflammation induced by postnatal EB, T and E exposure affects amino acid and lipid profiling, we performed Xevo TQ-S triple-quadrupole mass spectrometer with Biocrates p180 kit using the rat plasma. Acylcarnitines, glycerophospholipids, and sphingomyelines were significantly increased in the rats dosed with EB, T and E, and were associated with chronic inflammation. In addition, glycine, citrulline and tryptophan levels were altered during chronic inflammation. The metabolomics findings suggest that these metabolic changes may be associated with activated pro-inflammatory signaling, altered beta oxidation and may be potential biomarkers of prostatitis. As this study used a rat model, further experiments using human plasma samples will be necessary to confirm these findings.

1580 Surgical Implantation of Vascular Access Buttons Has No Impact on Male or Female Reproduction in Crl:CD1(ICR) Mice

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Reproductive toxicology studies in mice typically entail daily dosing for approximately 5 weeks for females and up to 8 weeks for males. As opposed to conventional routes of administration, such as oral gavage, daily tail vein intravenous administration is extremely difficult in the mouse and therefore, requires the use of surgically catheterized animals. The use of Vascular Access Buttons (VABs) presents significant advantages as the access button is fitted with a light weight magnetic cap that allows untethered access and permits social housing throughout the study. We present control group data from two independent definitive reproductive toxicity studies conducted per guideline ICH 4.1.1 in male and/or female Crl:CD1(ICR) mice implanted with VABs. Twenty two male or female mice were surgically implanted with jugular vein catheters using VABs. Animals were dosed daily with Phosphate Buffered Saline (PBS), pH 7.4, via bolus intravenous injection and later mated with naïve animals of the opposite sex for up to 15 days. Males were dosed for 28 days prior to mating and continuing through 1 day prior to euthanasia (at least 56 doses). Females were dosed for 14 days prior to mating and continuing through Gestation Day 7 (24-30 doses). Evaluated endpoints included: clinical signs, body weights, body weight gains, food consumption, gross necropsy and reproductive organ weights. In the VAB-females (mated with native males) or naïve female (mated with the VAB-males), the mean numbers of corpora lutea and implantation sites and intrauterine embryonic survival were evaluated. Parameters of reproductive performance included precoital intervals, mating, fertility, and copulation/conception indices. In addition, sperm parameters in males (motility, concentration, sperm production rate, and morphology) and estrous cyclicity in females were evaluated. The data demonstrated that the surgical implantation of VABs had no impact on male or female reproduction versus non-VAB animals, as compared to the Charles River Ashland historical control data. Therefore, the use of surgical implanted VABs is an acceptable approach for mouse reproduction studies.

1581 GSK2245035, a TLR7 Agonist, Does Not Increase Pregnancy Loss in Cynomolgus Monkeys

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GSK2245035 is a novel small molecule Toll-like Receptor 7 (TLR7) agonist that was in development as an immunomodulatory, intranasal treatment for allergic airways disease aiming to reduce Th2 and enhance Th1/Treg responses to aeroallergens *via* the local induction of type I interferons (IFNs). GSK2245035 demonstrated selectivity for potent release of type I IFNs compared to TNFα and IL-6 with dose dependent increases in the interferon inducible chemokine, IP-10 in the nasal compartment. Pregnancy is a highly complex condition requiring several phases of immune regulation. Implantation requires some pro-inflammatory processes including IFNs, ISGs (Interferon Stimulated Genes), TNFα, and IP-10. As intravenous administration of type I IFNs to pregnant monkeys have resulted in abortions, a pregnant monkey study was conducted with GSK2245035 to assess the risk for pregnancy. Pregnant monkeys were given 0, 3 or 30 ng/kg/week GSK2245035 intranasally at 50 µL/nostril once weekly, beginning on Gestation Day 20 (GD20) through delivery and from Day 7 postpartum (pp) through Day 63 pp. There was no GSK2245035-related effect on pregnancy or infant loss. Non-adverse GSK2245035related effects included an increased incidence of nasal discharge and increased mean body temperature at 30 ng/kg/week in maternal animals compared to controls, dose-dependent increases in IP-10 and IFNα at ≥3 ng/kg/week in maternal animals, and decreased IgM and IgG titers following KLH immunization in infants from maternal animals given ≥3 ng/kg/week. Even though there were systemic increases in cytokines as well as increased maternal body temperatures which together confirmed pharmacologic activity, systemic IFNα levels did not reach equivalent levels of recombinant type I IFNs that were abortifacient in monkey. These lower IFNα levels as well as the once weekly dosing and local intranasal administration vs daily subcutaneous or intramuscular dosing with recombinant type I IFNs, may be the reasons for the lack of effects on pregnancy; however, it should be noted that there was an undesired impact on immune function in the offspring.

1582 Toxicity and Microbiome Assessments of Triclosan as Determined in a Dose Range-Finding Study in Harlan Sprague Dawley Rats

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A dose range-finding study of Triclosan (TCS) was performed in rats to evaluate general toxicity and potential disruption of the gastrointestinal microbiome. TCS was examined due to its previous use as an antibacterial chemical in health and skincare products and concerns for continued exposure due to potential levels in surface and sewage waters. Ten dams per dose group were orally gavaged with 100, 250, 500, or 1000 mg/kg from Gestation Day (GD) 6-21 and through lactation until postnatal day (PND) 28. Pups were exposed *in utero* and dosed postnatally from PND 12-28, at the same level as their respective dams. Additional cohorts were included to collect biological samples for internal exposure levels and for microbiome assessments on the ileum, colon, and feces. Two corn oil control groups (2 mL/kg and 5 mL/kg) were also assessed for potential differences in microbial populations. Moribundity was observed at ≥ 500 mg/kg. Additionally, reductions in maternal body weight gain and food consumption were observed at ≥ 500 mg/kg. Reductions in pup body weight gain were observed at ≥ 250 mg/kg, but no treatment-related toxicity was observed at 100 mg/kg. Selected animals were also assessed for effect of TCS on taxonomic diversity and composition of the microbiota in feces. When examining the microbial population, the bacterial genus and species abundance in both dams and pups showed a significant change in the phyla level at 1000 mg/kg in the dams. Alterations at the genus level were noted in PND28 pup samples at the lowest dose (100 mg/kg) and TCS exposure differences in bacterial species were seen between dams and pups. These data suggest that TCS, at the exposure levels examined, is associated with both general maternal and pup toxicity at doses of ≥ 250 mg/kg and had impacts on the maternal and pup gastrointestinal microbiome at doses of 100 mg/kg and higher.

1583 Trichloroethylene Decreases Fetal Weight in Timed-Pregnant Wistar Rats: Differential Effects of N-acetyl-L-cysteine and Aminooxyacetic Acid on Modulating Trichloroethylene Toxicity

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Trichloroethylene (TCE) is an industrial solvent used as a metal degreaser and in synthesis of various chemicals. Although epidemiological studies associate TCE exposure with adverse pregnancy outcomes, mechanistic explanations for these associations are incomplete. We hypothesize that toxicity of TCE is due to its glutathione conjugation metabolism, which has been scarcely studied in pregnancy. In the present study, we exposed timed-pregnant Wistar rats to 480 mg TCE/kg/day from gestational day (GD) 6 to GD 16. Rats were exposed to vehicle alone, TCE alone, or TCE in combination with the glutathione metabolic pathway modulators aminooxyacetic acid (AOAA) at 20 mg/kg/day or N-acetyl-L-cysteine (NAC) at 200 mg/kg/day administered from GD 5 to GD 16. AOAA is an inhibitor of cysteine conjugate β-lyase (CCBL), which is important for metabolic activation of TCE to 1,2-dichlorovinylthiol (DCVT), a highly reactive and toxic metabolite. NAC is a precursor of reduced glutathione and can augment intracellular glutathione concentration and scavenge free radicals. TCE decreased fetal weight in the rats, an effect that was rescued by AOAA but not NAC pre/co-treatment, suggesting that TCE confers its toxicity to fetuses via CCBL activity and DCVT formation. However, enzyme activity assays indicated that AOAA was effective as a CCBL inhibitor in maternal kidney but not in maternal liver nor the placenta, suggesting that the reduction in fetal weight was independent of AOAA-sensitive CCBL metabolic activation of TCE in placenta and maternal liver, but may be mediated by kidney and possibly other organs. Whereas placental morphometric measurements did not differ between the control and TCE-only treatment group, the TCE + NAC group had larger basal zone area and width along with smaller labyrinth zone area and width compared to the TCE-only treatment group when normalized to total placenta dimensions. This morphology of the TCE + NAC group is similar to a developmentally delayed placenta. We propose that the effect of NAC could be due to its ability to augment synthesis of glutathione, a substrate for glutathione *S*-transferases that is upstream of DCVT in the TCE glutathione metabolic pathway. Efforts are underway to more fully understand the role of glutathione metabolism in TCE fetal toxicity.

1584 Dioxin Induced Adaptations at the Maternal-Fetal Interface

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The actions of dioxins, such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD), are mediated by the aryl hydrocarbon receptor (AHR), a ligand-dependent transcription factor. Dioxin-AHR interaction results in the transcriptional activation of target genes such as *Cyp1a1*. TCDD has been reported to affect pregnancy and possibly development of the hemochorial placenta. The purpose of this study was to investigate TCDD actions on pregnancy and placentation using the rat as a model. High TCDD exposures resulted in pregnancy failure; however, lower TCDD exposures resulted in prominent adaptations at the maternal-fetal interface. Gestation day (gd) 13.5 placentation sites from TCDD-treated pregnant rats exhibited deep intrauterine endovascular trophoblast invasion and uterine spiral artery remodeling, which was not observed in oil treated controls. Immunostaining for CYP1A1 in gd 13.5 placentation sites revealed specific activation of AHR signaling in endothelial cells lining small diameter blood vessels at the uterine-placental boundary (metrial gland) and in endothelial cells within the extraembryonic mesenchymal cores of the labyrinth zone but not in the junctional zone of the placenta. Single cell RNA-Seq analysis of the metrial gland of pregnant rats revealed a high level of expression of *Ahr* in endothelial cells; however, minimal expression in intrauterine invasive trophoblast cells. The importance of AHR and CYP1A1 in the placentation site adaptive responses to TCDD were investigated in *Ahr* and *Cyp1a1* null rat models. We found that TCDD-induced intrauterine trophoblast invasion and uterine spiral artery remodeling was AHR-dependent but not affected by CYP1A1. We next evaluated the importance of AHR signaling in maternal versus embryonic/extraembryonic tissues following TCDD treatment. The presence of AHR in maternal tissues was essential for TCDD-induced adaptations at the placentation site. Placentation site adaptations were not affected by embryonic/extraembryonic AHR expression. RNA-Seq, RT-qPCR, and immunostaining of the metrial gland revealed that TCDD dysregulated uterine natural killer (NK) cell specific transcript expression without affecting overall NK cell numbers, indicating that TCDD treatment affected the uterine NK cell phenotype. Uterine NK cells have a prominent restraining effect on intrauterine trophoblast invasion, which may be modified by TCDD. In summary, exposure to dioxin during pregnancy induces robust AHR-dependent structural changes within the uterine-placental interface. Most interestingly, our findings indicate that at least some of the TCDD effects on placentation are mediated through its actions on the mother and not directly on trophoblast cells. *Supported by NIH grants ES028957, ES029280; Sosland Foundation.*

1585 Sex Differences in Benzo[a]pyrene Metabolites and Gonadal Transcriptomal Changes in Mouse Embryos after Oral Dosing to Pregnant Dams

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Polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene (BaP) are ubiquitous pollutants formed during incomplete combustion of organic materials. We previously reported that maternal oral dosing with BaP from embryonic day (E) 6.5 to E15.5 dose-dependently decreased ovarian follicle numbers in F1 female mice and testicular spermatid counts in F1 males, with the developing ovaries being more sensitive than the developing testes. We also previously showed that exposure to BaP destroys germ cells in cultured E13.5 mouse ovaries, but not in cultured E13.5 testes. The goals of the present study were 1) to measure BaP metabolites in embryos and placentas after oral dosing to pregnant dams and 2) to measure the impact on the gonadal transcriptomes. We hypothesized that greater effects would be found in the developing ovary than the testis. Pregnant C57BL/6J female mice were orally dosed via pipet with 0 (sesame oil), 0.2, or 2 mg/kg/d BaP from E6.5-E11.5. E13.5 gonads from the 0 and 2 mg/kg/d BaP groups were processed for RNA sequencing. Whole embryo (minus gonads) and placentas were harvested from all 3 dose groups for the measurement of BaP metabolites by HPLC. The sum of BaP metabolite concentrations increased with dose in the embryos and placentas ($P=0.006$), and concentrations were higher in female than male embryos ($P=0.034$). The major BaP metabolites detected in both male and female placentas were 3(OH)BaP and 9(OH)BaP, while in both female and male embryos, BaP-7,8-diol and 3(OH)BaP were the major metabolites. Whole gonad RNA sequencing identified 33 differentially expressed genes (DEGs; FDR <0.05) in BaP compared to control ovaries, and 5 DEGs in testes. Seven Kyoto Encyclopedia of Genes and Genome (KEGG) pathways were statistically significant in ovaries (ribosome, oxidative phosphorylation, Huntington's, Alzheimer's, Parkinson's, phagosome, non-alcoholic fatty liver disease), while none were in testes. The results show that BaP metabolites are present in mouse embryos after oral dosing to the mother, that the concentrations are higher in female than male embryos and that gene expression is altered to a greater extent by transplacental BaP in the embryonic ovary than testis, supporting our hypothesis. *Supported by NIH R01ES020454.*

1586 Direct Comparison of Acute Toxicity and Endocrine-Disrupting Effects of Seven Bisphenols in *Danio rerio*

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Due to endocrine disrupting potential concerns, bisphenol A (BPA) has been replaced with structurally similar compounds. Recent studies have shown some of these alternatives have similar/greater toxicological concerns; however, there are only limited reports of concurrent testing of BPA and alternatives to determine their relative safety *in vivo*. Zebrafish (*Danio rerio*) is an ideal model for use in studies when high-throughput capability is needed. Therefore, we compared acute toxicity and endocrine disrupting effects of BPA and 6 other bisphenols (BPB, BPF, BPS, BPZ, BPAF, and BPAP) in early developmental zebrafish. Embryos were dechorionated and exposed to 7 concentrations (ranging 0.05-500 ppm; n=6, 25/replicate) from 4 to 120 hours-post-fertilization (hpf) and full spectrum lethality curve was generated. In order to compare the endocrine disrupting potential of different bisphenols, three concentrations (LC10, LC10/10, and LC10/1000) was subsequently used in an 8-days exposure protocol (4-hpf to 8-dpf) to investigate potential effects on the hypothalamic-pituitary-gonadal axis. Bisphenols resulted in varying degrees of general toxicity with LC50 values ranging from 1.83 to 209 ppm in the following order: BPAF<BPZ<BPAP<BPB<BPA<BPF<BPS. Malformations caused by BPF exposure was most prominent. Gonadotropin-releasing hormone 3 (*Gnrh3*) mRNA expression was significantly downregulated with BPS at the lowest concentration tested (113 ppb) only vs. controls, showing low-dose effect. Trend towards decrease in *Gnrh3* expression was also observed in all BPA concentrations (4.4 ppb-4.4 ppm) vs. controls although not statistically significant. BPF caused significant increase of gly-

coprotein hormone α subunit (*Cga*) mRNA expression in groups treated with medium concentration (1.3 ppm) vs. controls. Luteinizing-hormone β subunit (*Lhb*) mRNA expression was increased with BPB, BPS, and BPZ at the highest and with BPF at the medium concentration vs. controls. Follicular-stimulating hormone β subunit (*Fshb*) mRNA expression was upregulated only with two lower concentrations of BPAP, showing low dose effect. Our findings present added evidence that BPA alternatives are not necessarily safer than BPA. Low-dose effects were observed with BPS and BPAP on key regulatory genes of the reproductive-axis, warranting further investigation. Moreover, *in vivo* models such as zebrafish could be used to implement comprehensive high-throughput testing in future selection of safer alternatives.

PS 1587 Microcystin-Induced Transgenerational Reproductive Toxicity and the Role of Epigenetic Modifications

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Persistent ecosystem damage perpetuates the human and animal health risk of microcystin-producing harmful algal blooms. Well-established acute hepatotoxins, microcystins (MCs) are known serine/threonine protein phosphatase inhibitors and can alter cell signaling, causing a wide variety of clinical signs including death. Studies highlight the reproductive system as an additional target organ and of the over 100 MC congeners, MCLR is the most commonly detected and studied. Multigenerational studies in mammals and aquatic species identify offspring of exposed parents have altered neurodevelopment and growth. In the alternative model *Caenorhabditis elegans* (*C. elegans*) MCs induce germline apoptosis at P0, making it an ideal model to study the generational epigenetic impact of MCs. In our studies L4s are exposed to a range of environmentally relevant concentrations MCLR (0.1-100 µg/L) for 48 hours with food in liquid. The *C. elegans* strains N2 (wildtype), TY2441(yls34 (Pxol-1::GFP+rol-6 (pRF4))) and WH371(ojIs50 [pie-1p::GFP::air-2 + unc-119(+)]) are used. Exposed P0 worms are recovered and each adult generation is evaluated for multi- (F1) and trans- (F3) generational toxicity without further MC exposure. In a general toxicity screen, N2 worm size (length, width, area), eggs laid and brood size of F1 and F3 were not significantly altered after MCLR exposure. Ancestral 0.1 and 100 µg/L MCLR exposure significantly increased germline apoptosis in N2 F1 (18% increase, $p<0.05$) and F3 (40% increase, $p<0.01$) generations and embryonic lethality in the F3 generation (fold change of 2, $p<0.05$). Epigenetic changes evaluated to explain the multi- and transgenerational MCLR-induced reproductive toxicity include chromosomal remodeling, where N2 F3 worms ancestrally exposed to 0.1 and 100 µg/L MCLR have a significant increase in chromosomal clumping (50% increase, $p<0.01$), but do not have an increase in missegregation based on a screen using the GFP::xol-1 strain which identifies GFP positive male embryos. Initial studies using immunohistochemistry identify histone marks H3S10p and H3K9me3 as reduced in N2 F1 late diakinesis oocytes, and using the GFP::air-2 strain, the kinase responsible for H3S10 phosphorylation was also reduced in F1 and F3 maturing oocytes. Together these preliminary results suggest MCLR may cause transgenerational reproductive toxicity through altered histone modifications.

PS 1588 Multigenerational Reproductive Effects of Perfluorooctane Sulfonic Acid in *Daphnia magna*

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Perfluorooctane sulfonic acid (PFOS) is a widespread environmental contaminant routinely detected in drinking water and human serum samples across the US. Since PFOS has also been detected in human follicular and amniotic fluids, female germ cells and developing embryos are subject to direct PFOS exposure. To test the hypothesis that germ cell and early developmental exposure to PFOS produce negative reproductive effects across multiple generations, age-matched, sexually mature *Daphnia magna* were individually exposed to 10µg/L PFOS, 100µg/L PFOS, or standard culture medium for 10 days. These exposure concentrations were designed to produce internal PFOS concentrations relevant to human levels. The exposure period was long enough for internal concentrations to stabilize and for at least one cycle of oocyte maturation to be completed. After this time, F_1 neonate (<24 hours) offspring were pooled within treatments and twelve offspring per treatment randomly selected and maintained individually in standard culture conditions for 21 days. The third broods of the F_1 generation were used to establish the F_2 generation, which was maintained similarly to the F_1 generation. Each generation was monitored daily with counting and removal of live and dead offspring, as well as aborted embryos. PFOS exposure to the P_0 generation caused an insignificant decrease in total live offspring produced at 100µg/L. However, using a generalized linear model, the total number of nonviable (i.e. sum of dead neonates and aborted embryos per adult) was significantly increased at 100µg/L PFOS. In the F_1 generation, prenatal/neonatal PFOS exposure resulted in a significant increase in nonviable offspring produced at both 10µg/L and 100µg/L. In the F_2 generation, the rate of survival to adulthood was decreased in both PFOS treatment groups. These preliminary results suggest that early life exposure to PFOS may negatively impact adult reproductive health.

PS 1589 The Pesticide Temefos Decreases Sperm Quality and is Metabolized in Reproductive Tissues at the Recommended Safe Concentrations in Rats

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The organophosphorus (OP) pesticide temefos (Tem) is used in the control of vectors that transmit diseases in Mexico. The LOAEL of Tem in male rats according to the WHO is 100 mg/kg/day/44 days (Category III) and 0.9 mg/kg/day/7-90 days for EPA (Category II). *In vivo* studies demonstrated the acetylcholinesterase (AChE) inhibition and DNA damage, and studies in HepG2 cells, which are metabolically active have reported the formation of micronuclei. Tem is unstable and previous studies have suggested that Tem is metabolized by mixed-function oxidases; some oxidized metabolites have been detected in rats, such as Tem-sulfoxide, Tem dioxon sulfoxide, Tem dioxon sulfone, and hydrolyzed products including thiodiphenol (TDP), TDP-sulfoxide, and TDP-sulfone (known as bisphenol S-BPS, considered as an endocrine disruptor). Due to the limited information of the toxic effects of Tem on the reproductive system, our interest was to evaluate the effects of Tem exposure (1 or 100 mg/kg/d/7 d, gavage) on AChE activity, sperm quality, peroxidative damage (MDA concentration), and DNA integrity (by SCSA) in spermatozoa collected from the epididymis-vas deferens, as well as the presence of Tem and its metabolites in different tissues (by HPLC-DAD-MSD) of male Wistar rats euthanized 1-hour post treatment. At 1 mg/kg/d, AChE activity decreased (22-29%) from the 3rd day, and no adverse effects on sperm cells were observed. At 100 mg/kg/d, AChE was inhibited (70-79%) from the 3rd day; 41% of the animals died after the 5th dose, and a decrease (11.5%) in the liver relative weight was observed. Sperm motility and viability decreased (30 and 8%, respectively), lipid oxidation increased (25%), and a poor sperm chromatin condensation was observed. Tem was widely distributed in a tissue-dependent fashion and it was 17-fold higher in the epididymis than in testis. The order of Tem metabolic activity was: liver> testis > epididymis. These results suggest that the liver and male reproductive system are targets of Tem that can cause fertility problems. Very importantly, the proposed safe concentrations (LOAEL) of Tem should be reevaluated in toxicological studies in different tissues.

PS 1590 Bisphenol S Impairs Human Cytotrophoblast Syncytialization through Competitive Epidermal Growth Factor Receptor Binding

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Bisphenol S (BPS) is an endocrine disrupting chemical and the second most abundant bisphenol detected in humans. Recently, we have demonstrated that *in utero* exposure to BPS reduces the endocrine capacity of the ovine placenta by reducing binucleate cell number. During placentation, ovine trophoblast cells undergo homokaryotic fusion to form binucleate trophoblast cells, a process similar to that of human syncytiotrophoblast formation. Syncytiotrophoblasts are essential for hormone production and nutrient exchange. Given that BPS reduces binucleate cell number in the ovine epitheliochorial placenta, we hypothesized that BPS will also impair trophoblast syncytialization in the human hemochorial placenta. To test this hypothesis, we used human cytotrophoblast cells (hCTBs) that were isolated from healthy placentas (n=6; IRB#15-484) derived from elective C-section pregnancies at term. hCTBs were cultured for 96 h with: 1) Vehicle (0.1% DMSO), 2) BPS (200 ng/ml), 3) human epidermal growth factor (hEGF, 10ng/ml), or 4) BPS+hEGF. hEGF was used as a syncytialization-induction signal and served as a positive control. Exposure to BPS reduced hEGF-mediated syncytialization rate (Vehicle: 11.58±1.17%, BPS: 11.23±1.17%, EGF: 21.71±1.17%, BPS+EGF: 11.97±1.17%; $P<0.001$). We additionally tested whether BPS could inhibit the EGF response by blocking EGFR phosphorylation in MDA-MD-231 breast cancer cells due to their high expression of EGFR. Cells were exposed to the same aforementioned treatments and phosphorylation of EGFR (pEGFR) and AKT (pAKT), a downstream signal to EGFR, were assessed by western blotting. As expected, hEGF upregulated pEGFR and pAKT (15.2 and 10.1-fold, respec-

tively, compared to the vehicle), but hEGF+BPS reduced pEGFR and pAKT (5.1 and 1.2-fold, respectively,compared to the hEGF group). To test if BPS directly competes with hEGF for the EGFR binding site we used an EGF/EGFR AlphaLISA assay, and demonstrated that BPS blocked EGF binding in a dose dependent fashion, with a reduction of up to 58%. This is the first study to demonstrate that BPS can 1) prevent EGF-mediated trophoblast syncytialization, 2) block EGFR phosphorylation, and 3) compete with EGF for receptor binding. Given the role of EGFR in placental development, including trophoblast proliferation and differentiation, BPS interference may result in placenta dysfunction. *Supported by NIEHS R01 ES027863 to A.V-L.*

1591 Effects of Pyrethroid Insecticides on Fetal Growth, Neurodevelopment, and Placental Function

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Prenatal exposure to pyrethroid insecticides has been associated with increased risk for neurodevelopmental delay and low birth weight in children. Despite widespread exposure, there is a significant gap in knowledge about the mechanisms by which pyrethroids alter fetal growth and neurodevelopment. To address this question, CD1 mouse dams were treated with 10mg/kg alpha-cypermethrin or corn oil vehicle via oral gavage from embryonic days 11 to 14 (E11-E14) or E5-E18. Shorter-term exposure reduced fetal body weight and delayed tangential migration of GABAergic progenitors into the cortical plate of E14 offspring embryonic brain. Longer-term exposure also delayed GABAergic progenitor migration, as shown by a higher density of GABAergic interneurons in E18 cortical white matter. To investigate the mechanisms responsible for impaired neuronal migration, migrating GABAergic progenitors were isolated via laser capture from E14 brain tissue and analyzed via RNAseq. Differential gene expression in response to cypermethrin exposure was assessed using Ingenuity Pathway Analysis, which implicated alterations in several critical biosynthetic and metabolic pathways as major contributors to cypermethrin's effects. To investigate whether these effects are due to direct exposure to the embryonic brain, levels of cypermethrin were measured via GC-MS in maternal serum and amniotic fluid. Levels of cypermethrin in amniotic fluid were found to be below the limit of detection, implicating an indirect mechanism by which cypermethrin alters fetal growth and neurodevelopment. Analysis of placental tissue revealed that cypermethrin exposure altered multiple functional domains; in particular, expression of genes responsive to oxidative stress, including Hif-1alpha, Sod1, and Txrnd1. Despite this, cypermethrin exposure did not significantly affect placental weight. In conclusion, these studies suggest that maternal cypermethrin exposure impairs fetal growth and development of the GABAergic system in the embryonic brain. Furthermore, indirect placental mechanisms may play a significant role in the effects of maternal cypermethrin exposure on offspring development.

1592 Pre- and Postnatal Subchronic Toxicity Studies of Boric Acid in the Harlan Sprague Dawley Rat

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Boron (B), administered as boric acid, has previously been shown to induce developmental and reproductive toxicity, and the USEPA oral reference dose (RfD) is based on reduced fetal weight in the rat ($BMDL_{05}$=10.3 mg B/kg/day). Recent epidemiological studies on boron suggest that infants may be a potentially sensitive subpopulation; however, there is no data from animal studies to assess toxicity concerns for this age group. We evaluated the effects of pre- and postnatal boric acid exposure on development and select male reproductive endpoints in the Harlan Sprague Dawley rat. In the range-finding study, time-mated females were dosed with vehicle (deionized water), 5, 10, or 20 mg B/kg/day (administered as 0, 28.6, 57.1 or 114 mg boric acid/kg/day) via gavage from gestation day (GD) 6 to 21; pups were dosed via gavage from postnatal day (PND) 1 to 28. In 20 mg/kg pups, umbilical hernias were observed, and pup weights were 18% less than control offspring on PND 28. Based on these findings, doses of 0, 4, 8, 12, or 16 mg B/kg/day (administered as 0, 22.9. 48.8, 68.6, 91.5 mg boric acid/kg/day) were selected for the main study, which included subchronic exposure in males from PND 28-118. During lactation, there was increased pup mortality at 16 mg/kg, a dose-dependent increase in umbilical hernias (resolved by PND 28), and lower PND 28 pup weights than the controls (4%, 6%, 12%, and 25% lower at 4, 8, 12, and 16 mg/kg, respectively). Terminal body weights partially rebounded but remained 12% lower than controls at 16 mg/kg on PND 118. Organ weight changes were limited to lower pituitary, adrenal gland, and ventral prostate weights (absolute and relative), which occurred in a dose-dependent manner. No treatment-related effects were present in sperm parameters on PND 118. Plasma boron concentrations on PND 4, 28, and 118 males revealed a dose-proportional increase with the concentration in the controls less than or equal to 3.4% of the concentration of the lowest dose at any timepoint. Overall, the findings of reduced body weight suggest that exposure to boron during early postnatal developmental may result in developmental delay and highlight the importance of evaluating toxicity during the early postnatal period.

1593 Early Exposure to the Hypolipidemic Agent Rosuvastatin and Its Effects on the Development and Fertility of Female Wistar Rats

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Statins are drugs used to reduce cholesterol. The use of statins by young people is increasing due to poor eating habits and sedentary lifestyle. Studies conducted in our Laboratory reported that exposure to rosuvastatin impaired reproductive function of male rats. The effects on the female reproduction remains unknown. This study aimed to evaluate the effects of rosuvastatin in reproductive development and function of female rats exposed to this drug since pre-puberty. Female Wistar rats were allocated into three experimental groups: control, treated with saline (vehicle); and R3 and R10 groups, which were treated with rosuvastatin at doses of 3 or 10 mg/Kg/Day. Treatments were performed daily, gavage, starting on postnatal day / PND 22 and ended at two different ages: at puberty (around PND 42) and at adulthood (PND 75), when the rats were euthanized in estrus and evaluated for body and organ weights, and steroid hormone levels. At adulthood part of the rats from each group was tested for sexual behavior and mated for fertility assessment. Pregnant females were euthanized on gestational day 20. The following parameters were also evaluated: age of puberty onset; estrous cyclicity starting on PND 60; uterine motility, both on gravidic and non-gravidic rats. Additionally, the uterotrophic assay was performed. For this, female Wistar rats were allocated into the same experimental groups and the treatments were associated with estradiol benzoate (0.4mg/Kg/day) or corn oil (vehicle). Treatments were performed from PND 21 until PND 23, and rats were euthanized on PND 24 for measurement of uterus weight. Statistics: ANOVA and Kruskall-Walis, $p < 0.05$. Puberty onset was not altered by juvenile rosuvastatin exposure. However, R10 group showed shorter reproductive cycles than control group. Body and organ weights were not affected by the treatment, except for liver and hypophysis of animals at PND 42, which were reduced in R10 group. Hormone levels were similar among groups at all ages. On the other hand, uterine motility, either in non-gravid and gravid uterus showed alterations in R3 and R10 animals. Rosuvastatin was also associated with altered reproductive performance, once R10 animals were less receptive during sexual behavior and placental weight was decreased compared with control group. Rosuvastatin exposure in the uterotrophic assay did not present signs of estrogenic or anti-estrogenic effects. Our results showed that pre-pubertal exposure to rosuvastatin altered female rat reproductive development and function, raising concern for women reproductive health after using this statin since young ages. *Funding: CNPq.*

1594 Differential Sensitivity to Novel Stimuli and *d*-Amphetamine following Adolescent Methylmercury Exposure

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Methylmercury (MeHg) is an environmental neurotoxicant known to alter dopamine-mediated behaviors. The appropriate development of dopamine systems is essential to behaviors related to attention-deficit/hyperactivity disorder, including sustained attention and short-term memory. The current study modeled sustained attention and memory following exposure to MeHg throughout adolescence, the period of final dopaminergic development, using a mouse model. Mice were exposed to 0, 0.3, or 3ppm MeHg throughout adolescence and trained in a visual signal detection task in adulthood. Sustained attention and memory were probed with novel tactile and visual stimuli. Behavior of all animals, across MeHg exposure groups, was impaired significantly by the tactile stimulus. However, animals exposed to 3ppm were

unaffected by the visual stimulus while 0 and 0.3ppm animals were disrupted. In order to examine this further, animals were administered 0.3mg/kg *d*-amphetamine and probed with the visual stimulus. Animals exposed to 3ppm MeHg were differentially sensitive to *d*-amphetamine under the visual stimulus. This differential sensitivity supports hypotheses that adolescent MeHg exposure alters dopamine neurotransmission and is in line with observations of behavioral inflexibility following adolescent MeHg exposure.

1595 Dietary Route of Exposure for Rabbit Provides a Reliable, Relevant, and Conservative Characterization of Developmental Toxicity

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Rabbit prenatal developmental toxicity studies via the dietary route of exposure have been conducted by Corteva Agriscience heritage companies since 2008. Although rabbits can be sensitive to feed palatability and typically display variability in gestation feed consumption, this route of exposure has been optimized using a palatability rangefinder, a daily feed allotment instead of *ad libitum* access, and the use of a flavor additive to improve palatability of test diets. Dietary exposure is most relevant for modeling human pesticide residue exposures and provides a more continuous exposure based on toxicokinetically measured blood concentrations throughout the developmental period. Achieved doses and resulting NOAELs via dietary exposure in the rabbit for four recently registered pesticides (Rinskor, Sulfoxaflor, Inatreq, and Arylex) were in line with the overall toxicology data package. Furthermore, toxicokinetic data from these dietary rabbit studies demonstrated fetal exposure in all cases. Despite the numerous advantages, logistical challenges associated with this route of exposure have prevented most laboratories from attempting to perform dietary developmental toxicity studies in rabbits. Charles River Laboratories, Ashland recently generated historical control data (HCD) with the feed allotment model used by Corteva. Groups of 50 time-mated Hra:(NZW)SPF female rabbits were provided 125 or 150g flavored feed/day during gestation. Mean maternal body weights, gravid uterine weights and net body weights were comparable to historical data for both groups of rabbits given the different allotments. Intrauterine growth, survival and fetal morphology (fetal body weights, sex ratios, pre/postimplantation loss, and incidence of fetal visceral, external, and skeletal anomalies) were also comparable to HCD for *ad libitum* fed females. These data support the use of the laboratory's existing HCD for the interpretation of data obtained on rabbit dietary studies using a set daily feed allotment. Together, these data demonstrate that dietary exposure in rabbits provides reliable and human-relevant exposures that can be used to characterize the potential for an agrochemical to cause developmental toxicity.

1596 Proposal for Study Designs Based on Results of Juvenile Toxicity Studies in Rats

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The ICH S11 guideline Step 2b (2018) clarifies the endpoints that should be implemented when juvenile animal studies (JAS) are conducted. While Step 2b provides details on study methods, appropriate approaches and endpoints must be set on a case-by-case basis. In this presentation, we compare JAS in SD rats conducted at our facility since 2011 with the study design in Step 2b and propose designs we find most appropriate, focusing on 3 main points. Allocation: We used 4 allocation methods in the past JAS. Each method had both advantages and disadvantages, but the most important factor was body weight. In 11 of the 24 studies, a statistically significant difference was noted in body weight among groups at pre-dosing. Of the 11 studies, 8 were definitive JAS, and past results showed that an increase in the number of pups per group was not related to a decrease in bias (e.g. dose-range finding study: 6 to 8 pups/group, definitive study: 10 to 20 pups/group). Preweaning body weight is an important factor as it affects viability and subsequent growth, and nonuniformity in body weight among the groups at pre-dosing affects evaluation of development, one of the purposes of JAS. Accordingly, although it is a more complicated method, we propose allocation based on body weight of pups at allocation. First dosing age: Step 2b states that the first dosing age of pups should be equivalent to the youngest intended target human age. In 54% of 24 JAS conducted at our facility, dosing started from postnatal days (PND) 4 or 7. The current trend of the first dosing age is PND 7 since 80% of JAS conducted at SNBL started on PND 7 in the last 2 years. Neonates for humans is defined as 0-27 days old, and the corresponding time for humans is PND 0-9 for rats. We therefore propose PND 7 as an appropriate first dosing age when the clinical target is neonates in humans. Blood sampling: Although conventional preweaning blood sample collection is often conducted at terminal, we have established a sampling method from the submandibular vein that reduces the number of pups needed for toxicokinetics by approximately 45%. When we sampled 0.2 mL of blood once from PND 10 pups, returned them to their dam, and allowed them to nurse until weaning, no abnormalities were noted in clinical signs or body weight gain in comparison with historical control data. We therefore propose blood sampling from the submandibular vein in JAS in rats. Thus, if the design is set in consideration of the points above, more appropriate toxicity assessment becomes possible.

1597 Developmental Toxicity Studies of an AcetylCoA Carboxylase Inhibitor in Sprague Dawley Rats and New Zealand White Rabbits

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Acetyl-CoA carboxylase (ACC) catalyzes the first step of *de novo* lipogenesis (DNL). Pharmacologic inhibition of ACC has been of interest for therapeutic intervention in a wide range of diseases. Developmental toxicity studies were conducted with a systemically acting dual ACC1/2 inhibitor (PF-05175157) in rats and rabbits with doses up to 30 and 50 mg/kg/day administered from GD6-17 and GD7-19, respectively. In rats, there were no effects on maternal body weight and no effects on embryo-fetal survival. Lower gravid uterine weights corresponding to lower fetal body weights were observed in rats at 30 mg/kg/day. Malformations and variations were observed in rat fetuses at 15 mg/kg/day (bifid thoracic centrum) and 30 mg/kg/day (gastroschisis; digit malformations; meningocele with irregularly shaped brain; malformed limbs, digits, or ribs; variations in the development of the skull bones, vertebrae, ribs, and sternebrae; ossification delays of the hyoid, caudal vertebrae and hindlimb phalanges). In the rabbit, maternal effects included lower body weight gain and food consumption at 50 mg/kg/day. Lower rabbit gravid uterine weights corresponding to increased postimplantation loss and reduced fetal body weights were observed at 50 mg/kg/day. Rabbit fetal findings observed at 50 mg/kg/day included short tail, malformed vertebrae or ribs, variations in the development of the skull bones, vertebrae, ribs, and sternebrae. Based on the data from these studies, inhibition of ACC results in developmental toxicity in both the rat and rabbit.

1598 *In Vitro* Evaluation of Acetyl-CoA Carboxylase Inhibitor-Mediated Developmental Toxicity

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Acetyl-CoA carboxylase (ACC) catalyzes the first step of *de novo* lipogenesis (DNL). Pharmacologic inhibition of ACC has been of interest for therapeutic intervention in a wide range of diseases. PF-05175157, a systemically acting dual ACC1/2 inhibitor, caused developmental toxicity (fetal malformations and/or post-implantation loss) in embryo-fetal development studies in both the rat and rabbit. Additional studies in rat whole embryo culture [WEC], mouse embryonic stem cells [mESC], and zebrafish developmental toxicity assay were conducted to evaluate the utility of these *in vitro* assays in detecting ACCi-induced developmental toxicity. These are relevant models because ACC is expressed in rats and in mESC, and a zebrafish ortholog is present. Evaluation of PF-05175157 in the rat WEC assay revealed a concentration-dependent decrease in morphological scores for the majority of developmental structures, indicating the potential for a direct effect of PF-05175157 on the fetus. In the mESC assay, PF-05175157 showed high risk for teratogenesis. In contrast to the WEC and mESC assays, the zebrafish developmental toxicity assay did not predict the potential for ACCi-mediated developmental toxicity. This was not due to lack of zebrafish exposure, as PF-05175157 was detected in zebrafish in a dose-dependent manner. This work demonstrates that a battery of *in vitro* assays to screen compounds for developmental toxicity is more informative than any single assay.

1599 Gestational and Developmental Toxicity of Polychlorinated Biphenyls (PCBs)

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Polychlorinated Biphenyls (PCBs) are persistent environmental pollutants with endocrine disrupting properties. Among 209 different congeners, PCB126 is the most potent dioxin-like toxicant which displays numerous adverse effects on human health, including female reproductive health. Our hypothesis is that prenatal exposure of PCB126 causes reproductive and de-

velopmental toxicity in rats. To test this, 12 timed pregnant Sprague Dawley rats (dams) were used. Dams received a single IP dose of corn oil (5mL/kg) or different doses of PCB126 (0.2, 1, 5µmol/kg) on gestation day (GD) 12 and were necropsied after 6 days on GD 18. PCB126 exposed dams showed significant weight loss compared to control dams. PCB126 significantly increased relative liver weight ($p<0.05$) and decreased relative thymus weight ($p<0.05$) compared to controls. It also caused mild to moderate macrovesicular vacuolation in the portal zone area of the liver (H&E stain). PCB126 exposed dams had low glycogen (PAS stain) in the liver. While control rats had no significant liver pathology. PCB126 significantly increased the expression of xenobiotic enzyme of CYP1A1 in livers compared to controls. In addition, serum cholesterol level was significantly increased and non-esterified fatty acids (NEFAs) was significantly decreased in PCB126 exposed dams. Significantly increased serum estradiol levels and significantly reduced plasma leptin level were measured in PCB126 exposed dams. PCB126 not only affected the pregnant dams but also the placentas and fetuses. It significantly decreased placental weight and caused necrosis in the placenta. It increased the thickness of the labyrinth zone in the placenta which may lead to intrauterine growth retardation (IUGR). It also increased the thickness of the basal zone in the placenta which may cause hormonal imbalance in mothers and fetuses. PCB126 significantly reduced number of viable fetuses and increased number of non-viable fetuses. It reduced the fetal body weight and increased implantation sites (sign of miscarriage) possibly due to observed dysregulation of estradiol homeostasis. The fetal sex ratio was skewed towards males in PCB126 exposed groups compared to controls. Therefore, our results show that even short time (6 days) *in utero* exposure to PCB126 impaired successful embryo development and produced endocrine and hepatic disruptions in pregnant mothers. *Supported by NIEHS: P42ES013661.*

PS 1600 Targeting Nrf2 through Lactational Transfer of Sulforaphane: Implications for Respiratory Syncytial Virus Severity in a Neonatal Mouse Model

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Early-life exposure to air pollution is associated with lower respiratory tract infections in infants and children. Data from our mouse model demonstrate *in utero* exposure to particulate matter (PM) causes offspring immune suppression and dampened response to respiratory syncytial virus (RSV) infection. RSV infection is the leading cause of severe respiratory illness and infant hospitalization. These data underscore the need for safe and effective strategies for mitigating oxidant-driven infant immune suppression. The Nrf2 antioxidant response plays an important role in oxidant-driven RSV pathogenesis, as well as PM-induced oxidative stress. Sulforaphane (SFN), a naturally occurring phytochemical found in cruciferous vegetables, targets Nrf2 to reduce oxidative stress. While this strategy has been employed in clinical trials and animal models, there are currently many unknowns in regards to Nrf2 activation early in life. Thus, our overall objective was to determine if maternal SFN supplementation via lactational transfer of SFN and/or its conjugates provides protection against neonatal RSV infection. Purified SFN, was incorporated into the diet of C57BL/6 female mice in 1-g aliquots of peanut butter at 200, 400 and 600 ppm for 3 days and compared to the vehicle control. Hepatic Nqo1, a prototypical Nrf2-regulated gene, showed a dose-dependent increase in expression, yet only reached statistical significance in the 600-ppm group. In subsequent experiments, SFN was incorporated in the maternal diet at 0, 300 or 600 ppm from delivery through postnatal day 5, based on the day of our neonatal RSV infection, when breast milk and plasma from dams and pooled litters was collected. Measurement of SFN and conjugated metabolites is ongoing using a modified HPLC-isotope dilution mass spectrometry method. Further evaluation of Nrf2 induction in dams and offspring and ultimately suppressed RSV infection will be completed. In conclusion, initial results indicate short-term maternal SFN supplementation enhances mRNA expression of Nqo1 and has potential implications for offspring protection during important windows of susceptibility. Future work will investigate the dual interaction of PM-enhanced RSV infection.

PS 1601 Assessing the Multigenerational Impact of Dietary Exposure to Benzo[a]pyrene

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Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (BaP), are implicated in neural tube defects, growth deficits, cardiovascular toxicities, endocrine disruption, childhood cancers and infertility in offspring of exposed parents. However complete understanding of the molecular mechanisms for the developmental and potential multi/trans-generational effects associated with PAH exposures is lacking. Our previous work has revealed significant multigenerational effects of BaP dietary exposure on survival and developmental deformities in AB strain zebrafish larvae. The goal of this current study is to characterize the transcriptomic and epigenetic changes associated with preconceptional exposure to BaP. To accomplish this, 5D strain zebrafish were fed 78.3 or 708 µg BaP/g diet (measured) at a rate of 1% body weight twice/day (1.6 or 14 µg BaP/g fish/day) for 21 days. At the end of the 21-day parental exposure, fish were spawned using a crossover design, and the effects in F1 generation were monitored. In contrast to our previous findings, we did not observe any developmental effects in this study, suggesting that the AB zebrafish line may be more sensitive to exposure to BaP. However, a significant reduction in larval motility (96 hpf) was observed in the offspring of exposed fish. Furthermore, F1 fish were significantly shorter in length at 1 and 3 months of age. These results demonstrate that dietary BaP exposure causes adverse developmental outcomes to multiple generations that may be strain related. *Research is supported by NIEHS 1R21ES030154.*

PS 1602 Atrazine Exposure Produces Same Major Metabolites as Mammals Along with Adverse Developmental Effects

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Atrazine (ATZ) is a triazine herbicide that is the second most commonly used in the United States and heavily applied to crops in the Midwestern US. ATZ is highly mobile in soils and contaminates potable water sources. Due to contamination concerns, the US EPA has set the maximum contaminant level in potable water sources at 3 parts per billion (ppb;ug/l). Depending on the time of year and sampling location, water sources often exceed this limit. ATZ has a long half-life and has also been implicated as an endocrine disrupting chemical in multiple species. The current study used a biomedically relevant model, the zebrafish, to test the hypothesis that developmental ATZ exposure generates metabolites similar to those found in mammals and alters morphology and behavior in developing larvae. Adult AB zebrafish were bred, embryos were collected, and exposed to 0, 0.3, 3 or 30 ppb ATZ from 1 to 120 hours post fertilization (hpf). Targeted metabolomics analysis found that zebrafish produce the same major metabolites as mammals: desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine with ATZ exposure. Morphology measurements show a significant increase in mean head width in the 0.3 ppb treatment group ($p<0.05$). No change in total length was found between treatment groups ($p>0.05$). The visual motor response test detected hypoactivity in the 3 and 30 ppb treatment groups with distance moved ($p<0.05$) and slowest acceleration ($p<0.05$). These findings suggest that a single ATZ exposure during early development generates metabolite profiles similar to mammals and leads to morphology and behavior alterations supporting ATZ as a developmental toxicant.

PS 1603 Holistic Models for *In Vitro* Embryotoxicity Testing: Do We Need Standardized Morphological Endpoints and Terminology?

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To address prenatal developmental toxicity (PDT), the OECD 414 is the regulatory required study for the registration of new chemicals. Alternative methods are under evaluation to be used in test batteries or IATAs. Holistic models with their complex physiological organized organ anlagen, like the zebrafish embryotoxicity assay (ZETA), provide the opportunity to cover various mode of actions of PDT. The ZETA is in broad use for screening purposes in development of new chemicals. For practical reasons morphological endpoints are primarily used, like in the related *in vivo* test. To assess the fetal development in rats about 300 morphological parameters with harmonized terminology are used. Even the zebrafish does not offer as many morphological endpoints, in most protocols less than 20 parameters are used and no broadly accepted protocol is available. To investigate the potential consequences of non-harmonized protocols we investigated 2 chemicals with the ZETA in three test facilities. In general, the culture conditions were comparable, but the morphological scoring was based on 17, 18 or 38 endpoints. The terminology differed in almost all parameters. Only 8 parameters could be linked to comparable morphological endpoints. The NOEC were reproducible in the different test facilities and ranged between 1-4 µM for Ketoconazole, and 10-12.5 µM for Tebuconazole. But the treatment related morphological alterations determining the LOEC differed: Ketoconazole - brain, jaw, or nasal cavity; and; Tebuconazole - heartbeat, heart edema, or brain. Thereby, the pattern of

morphological alterations for the test substances was not reproducible. The prediction of PDT of the test substances in mammals as well as the detection of the craniofacial alteration expected for the investigated azoles was only determined by the test facility using the comprehensive scoring system. Even the morphological scoring provides only limited conclusion of the mode of action of test substances, the potential of morphological endpoints of the ZETA is not always used yet to describe the test substance specific pattern of alterations. A broad and harmonized morphological scoring system, based on common terminology, is needed to reach interlaboratory reproducibility and increase the acceptance of this assay used as an example of holistic models.

1604 E-cigarette Vaping Liquids and a Flavoring Chemical Perturb Bone, Cartilage, and Vascular Development in Zebrafish Embryos

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Electronic cigarettes are an increasingly popular smoking device, especially among adolescents and youth. E-cigarettes are perceived to be a safer alternative to tobacco cigarettes leading to that pregnant mothers may replace tobacco smoking with e-cigarette use. However, whether these products have any side effects on the developing embryo has not been thoroughly evaluated. We tested cinnamon flavored e-liquids (with 12 mg/ml nicotine and without nicotine) and the flavoring chemical cinnamaldehyde on zebrafish embryonic development. We studied possible effects on hatching success, bone, cartilage and blood vessel development in 3-4 days old transgenic zebrafish larvae. Our study showed that the e-cigarette liquids significantly affected craniofacial bone and cartilage development. Exposure also resulted in structural malformations in intersegmental blood vessels. Hatching frequency was significantly delayed by vaping liquid exposure. Presence of nicotine in the e-liquid only slightly augmented the perturbations identified. Exposure to the favoring chemical cinnamaldehyde induced similar malformations as the vaping liquids, but at ten times lower concentration. Exposure to the humectant propylene glycol, present in e-liquids, did not cause any visible effect on hatching or bone development in zebrafish larvae at the concentrations tested. In conclusion, we show that exposure to cinnamon flavored e-cigarettes or the flavoring chemical cinnamaldehyde during embryonic development cause significant structural malformations in bone, cartilage and vasculature.

1605 Transcriptional Dysregulation in Zebrafish Larvae Exposed to Sodium Arsenite and Uranyl Nitrate

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Arsenic (As) and uranium (U) are naturally occurring contaminants frequently encountered in the Southwest United States. Human exposure to As and U comes in many forms including inhalation of contaminated dust and ingestion of contaminated food or water. Highlighting the Southwest US, exposure risk in the form of contaminated drinking water represents a major health disparity for many Native Americans living in tribal communities where barriers to clean drinking water exist. In these regions, As and U levels often exceed current EPA standards of 10 µg/L and 30 µg/L respectively, sometimes by orders of magnitude. For example, the Verde River in central Arizona has As levels documented up to 1300 µg/L. Morbidities resulting from high As or U exposure are well documented, but recent studies find that even moderate levels of exposure (levels at or near EPA limits), are associated with negative health-related outcomes. It is crucial that we understand the effects of environmentally relevant concentrations of As and/or U on an organism's health span. In these studies, we use the zebrafish (*Danio rerio*) as a vertebrate laboratory-test species and RNA-seq transcriptomics to test the hypothesis that As, U, or a mixture of the two will elicit significant differential gene expression in exposed organisms that include genes linked to human health. In our studies we used water containing inorganic arsenic in the form of sodium arsenite (75 µg/L and 750 µg/L), depleted uranium in the form of uranyl nitrate (30 µg/L and 300 µg/L), or a mixture of the two chemicals (75 µg/L and 30 µg/L respectively) to expose zebrafish embryos from 1 hour post-fertilization (hpf) to 96 hpf. Treatment solutions were not acutely toxic to embryonic zebrafish at the concentrations used. All test-subjects were collected at 96 hpf, total RNA was isolated, and RNA-seq libraries were prepared and sequenced at a depth of ~20 million reads/sample. Transcriptomic analysis showed significant differentially expressed genes for all chemical perturbations compared to the vehicle control with some occurring only at the high concentrations and others at both high and low concentrations. For example, low As altered 47 genes (29-up and 18-down), high As altered 143 genes (119-up and 24-down). Low U altered 16 genes (10-up and 6-down) and high U altered 296 genes (181-up and 115-down). Finally, concomitant exposure to low As and low U resulted in 77 differentially expressed genes (65-up and 12-down). We are further interrogating these data to determine if synergistic dysregulation of gene targets can be identified in the combined As and U exposure. *These studies were supported by NIH 1R25GM127199-01 (RSA), NSF HRD1712523 (PHK).*

1606 Assessment of Developmental Toxicity from Embryonic Exposures to the Emerging Contaminant Tris(4-Chlorophenyl) Methanol (TCPMOH)

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Tris(4-chlorophenyl)methanol (TCPMOH) is an emerging water contaminant with unknown etiology, but is believed to be a byproduct of DDT manufacturing. It is highly persistent in the environment and bioaccumulates, and is commonly found in marine mammals around the world. TCPMOH has also been measured in human breast milk which poses a risk for developing infants. However, almost no toxicity data is currently available. In this study, we investigate the hazard posed by developmental TCPMOH exposures using the zebrafish model (*Danio rerio*). Zebrafish (*Danio rerio*) embryos were exposed to 0, 1, or 5 µM TCPMOH beginning at 24 hours post fertilization (hpf). We assessed survival, overall growth, and morphology in embryos at 96 hours post fertilization. QPCR was utilized to assess gene expression of enzymes in phase I and phase II metabolic pathways. Developmental exposure to TCPMOH increased embryonic mortality in a dose-dependent manner. Pericardial edema, yolk edema, and craniofacial malformations were increased with TCPMOH exposure. Total growth was significantly increased in the 1 µM embryos, but significantly decreased in embryos exposed to 5 µM TCPMOH ($p < 0.05$). These results demonstrate that developmental TCPMOH exposures can negatively impact embryonic growth and development. Overall, this study demonstrates that the emerging contaminant TCPMOH is a toxicological concern, and further studies are required to elucidate the mechanisms of toxicity.

1607 Analyzing Estrogenicity of Bisphenol Analogues Using Transgenic Zebrafish

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Bisphenol A (BPA) has been widely used to make epoxy resin, polycarbonate plastics and other materials over the past decade. It is one of the most common environmental pollutants. Exposure to BPA has possible hazardous effects on the human brain, heart, liver, and blood, partly because of its estrogenic activity. BPA analogues have been synthesized to be considered as replacement molecules for BPA. These analogues need to be thoroughly evaluated for their estrogenic activity. We used transgenic *Tg(5×ERE:GFP)* zebrafish to study agonistic and antagonistic estrogenic effects of bisphenols *in vivo*. Three days old zebrafish embryos were exposed to a concentration range of BPA, BPC-CL, BPAF, BPC and BPE, incubated and imaged at day four by fluorescence microscopy. The fluorescence in images were quantified by ImageJ. The exposures to BPA, BPAF, BPC and BPE efficiently induced GFP expression first in the heart valves and at higher doses in the liver, whereas BPC-CL activated GFP expression in the liver and brain, although the GFP expression was reduced at the highest concentration. The GFP expression in fish was compared to estrogen response in reporter cells that express the zebrafish estrogen receptors driving expression of an ERE-luciferase reporter. BPC-CL preferentially activated esr2a, whereas BPA, BPAF, BPC and BPE preferentially activated esr1. We conclude that transgenic estrogen reporter fish in combination with estrogen reporter cells efficiently can be used to assess estrogenic capacity of environmental pollutants.

1608 Assessing Glutathione Utilization and Target Organs of Toxicity in a Complex PFAS Mixture

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Aqueous film forming foam (AFFF) is a major source of per- and polyfluoroalkyl substances (PFAS) contamination in the environment, especially for contaminated drinking water. AFFF contains a complex mixture of at least 100 different PFAS, hydrocarbons, and solvents. We recently received a legacy 3% formulation of AFFF (manufactured prior to 2002) and have demonstrated

that this mixture is more toxic than the most abundant PFAS in this formulation, perfluorooctanesulfonic acid (PFOS). PFOS is known to cause redox stress and perturb glutathione (GSH), the most abundant endogenous redox buffer; GSH also plays key roles in organogenesis and embryonic development. Monochlorobimane (MCB) has been found to be a robust and sensitive method to detect the spatiotemporal changes in GSH utilization *in vivo* and was used in the current study to identify target tissues of low, sublethal exposure concentrations of AFFF. Zebrafish (*Danio rerio*) embryos were exposed at 3 hours post-fertilization (hpf) to two concentrations of AFFF, and solutions were refreshed daily. At 48 hpf, embryos were washed and incubated in 20 μM MCB for 1 hour and imaged on a fluorescence microscope. A treatment of 1.1e-4% AFFF (n=15 fish) resulted in a 23% increase ($p < 0.008$) in the fluorescence of the embryo's body overall, but an 18% decrease ($p < 0.03$) of fluorescence in the gut. Doubling the concentration to 2.2e-4% AFFF (n=17) resulted in a 13% ($p < 0.02$) increase in fluorescence of the embryo's body and a downward trend in the fluorescence of the gut ($p < 0.2$), a similar trend as seen in the 1.1e-4% dose. Fluorescence of the body shows an inverted "U-shaped" response curve with increasing AFFF concentration suggesting that the larvae are more sensitive to disruption of GSH homeostasis at low concentrations. MCB staining indicates disruption of redox state and sensitive changes in GSH at lower AFFF concentrations. These redox perturbations in the gut precede impaired growth of the liver and exocrine pancreas observed at 96 hpf. *This work is supported in part by the UMASS Amherst Commonwealth Honors College Research Grant, R01ES025748, and R01ES028201.*

1609 Developmental and Nutritional Impacts of Maternal Preconception Exposure to PFOS

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Perfluorooctanesulfonic acid (PFOS) is a ubiquitous and persistent environmental toxicant that can be found in human urine and serum samples, including cord blood. In zebrafish (*Danio rerio*), embryonic exposures to PFOS have been shown to affect developmental endpoints and pancreatic organogenesis. However, the effects of maternal preconception exposure to PFOS on the offspring have not yet been examined. Therefore, using an evolutionary approach, this study aims to investigate and compare the effects of maternal preconception exposures to PFOS on morphological and nutritional endpoints in three different species: Zebrafish, *Caenorhabditis elegans* and *Drosophila melanogaster*. Adult female fish were exposed to either 0, 0.08, 0.14 or 0.25 μg/mL of PFOS for 7 days. Following exposures, female fish were paired with unexposed male and bred daily for two weeks. Hermaphrodite worms were exposed to 0, 10, 20 or 40 μM of PFOS. *D. melanogaster* female adults were exposed for 24 hours to either 0.2 ng/μL of PFOS or acetone prior to mating. Developmental and nutritional endpoints were measured. In all three models, exposures to PFOS caused developmental impairment. In *C. elegans*, adults exposed to PFOS (20 and 40 μM) had a significant decrease in broodsize and progeny number. Also, the offspring showed significant decrease in locomotive activity and size. In *D. melanogaster*, exposure to PFOS led to developmental delay in embryos and significant decreased weight in adults. In zebrafish, the first clutch of embryos from the 0.08 PFOS group had significant increase in yolk sac area that persisted to 120 hpf. By later collections, there was a significant decrease in length and pancreatic beta-cell area in embryos collected from the same group. Preconception exposures also influenced nutrient composition in *C. elegans* embryos, significantly decreasing triglycerides (20 μM group) and protein content (40 μM group). Interestingly, adult male and female offspring of maternally exposed *D. melanogaster* showed significant changes in cholesterol and triglycerides levels. Finally, PFOS was detected in zebrafish and *C. elegans* embryos. Together, these alterations seen in all three models suggest that preconception exposures to PFOS may impact oocyte maturation and nutrient loading in the offspring, possibly through maternal transfer of the toxicant into the eggs. *This work is funded by R01ES028201.*

1610 Bisphenol A-Induced Perturbations in Cellular Behavior during Murine Early Germ Cell Specification

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Through the environment, humans are exposed to a large number of different toxicants that have detrimental consequences on our health. A particular concern are the nature of these chemicals and their mechanism(s) of action and how they affect the normal physiology of different organs. Effects on the reproductive system, specifically the processes responsible for gametogenesis, are pertinent as any disruptions (genetic and/or epigenetic) have the potential to manifest as disease in the next generation and result in their trans-generational inheritance. Using an *in vitro* model of mouse primordial germ cell (PGC) development, we have examined the cell biological consequences of defined exposure to the chemical Bisphenol A (BPA). Exposure of Epiblast-like cells (EpiLCs) results in increased cellular proliferation, combined with reduced apoptosis and increased DNA damage. Despite transcriptome analysis of BPA-exposed EpiLCs suggesting the absence of large-scale transcriptional defects, this increase in proliferation persists during the transition into PGCLCs, indicative of an epigenetic basis for this altered behavior. Combined with the increased proportion of cells with DNA damage, this implies that BPA might result in the foundation of gametes with more germ cell precursors containing both genetic and epigenetic alterations. Our data provide novel insight into the aberrant effects of an abundant environmental toxicant during a crucial developmental stage that hitherto has been difficult to study.

1611 Validation of a Human Stem Cell-Based Biomarker Assay for *In Vitro* Developmental Toxicity Assessment

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Testing for developmental toxicity according to the current OECD guidelines requires large numbers of animals, making these tests very resource intensive and time-consuming as well as raising ethical concerns. In the last 20 years, several alternative *in vitro* assays have been developed but these often suffered from a low predictivity, the lack of mechanistic information and inter-species differences. To improve *in vitro* developmental toxicity testing, we have developed a human induced pluripotent stem cell (hiPSC)-based biomarker assay that follows the differentiation during early embryonic development. The hiPSC were directed to differentiate into the three germ layer specific cell types, hepatocytes, cardiomyocytes and neural rosettes. During differentiation, expression of the pluripotency marker OCT4 decreased, while expression increased for matured tissue specific markers MYH6 in cardiomyocytes, AFP in hepatocytes and PAX6 during neural rosette differentiation. Alterations in the expression pattern of these biomarker genes upon chemical exposure indicated perturbation of stem cell differentiation and thereby teratogenicity. To test the ability of the assay to identify developmental toxicity in differentiating hiPSC cells, we first tested 10 teratogenic and 5 non-teratogenic compounds at non-cytotoxic conditions during cardiomyocyte and hepatocyte differentiation. The assay classified thirteen compounds correctly as teratogenic or non-teratogenic substance with 80% sensitivity and 100% specificity. Next, the validation was extended with 40 additional reference compounds as described by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH S5 (R3)) and we were able to identify teratogenic compounds with different modes of action, including enzyme modulators, channel modulators and second messenger modulators. In conclusion, following the differentiation program by using selected biomarker is a suitable tool to quantify differentiation and could be used as a primary screen to identify chemicals with a high potential for DART.

1612 *In Vitro* to *In Vivo* Extrapolation for Developmental Toxicity Potency of Valproate Analogues

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To support implementation of alternatives to animal testing for regulatory decision-making for developmental toxicity, several case studies have been developed under the European Union ToxRisk project. In one case study, the teratogenic potency of ten valproate (VPA) analogues was investigated using an *in vitro* human induced pluripotent stem cell (iPSC)-based assay, the devTOX quick Predict assay (devTOX^{qP}). Previous work showed that the potency ranking from devTOX^{qP} was consistent with observed developmental toxicity potency *in vivo*. In this study, we applied *in vitro* to *in vivo* extrapolation (IVIVE) to evaluate the impact of pharmacokinetics and different modeling approaches on predicting relevant external exposure from *in vitro* developmental toxicity potential concentrations derived from the iPSC devTOX^{qP} assay. We used several pharmacokinetic models, including an open-source one-compartment model and both open-source and commercial physiologically-based pharmacokinetic pregnancy models. The IVIVE analysis estimated equivalent administered doses (EADs) that would result in maternal blood concentrations equivalent to the developmental toxicity potential and cytotoxic *in vitro* concentrations. The estimated EADs were compared to published lowest effect levels (LELs) from *in vivo* developmental toxicity studies.

Our preliminary results showed close agreement between EADs and *in vivo* rat LELs for two VPA analogues (within 3.5-fold), suggesting that the devTOX^{qP} assay and IVIVE approaches are suitable for quantitatively predicting *in vivo* developmental toxicity potential. This study highlights the importance of pharmacokinetic considerations in assessing a chemical's developmental toxicity potency based on *in vitro* assays. *This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.*

1613 Somatic Growth and Immune System Effects Observed Experimentally Following Pre- and Perinatal Exposure to Δ^9-THC

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Legalization of adult use of cannabis and its extracts in many states has raised public health questions about use during pregnancy. Here we discuss somatic growth and immune system effects examined in experimental studies investigating the potential of Δ^9-THC to cause developmental harm. Effects on embryo development and implantation, and later stages of embryo and fetal development were evaluated in various species using *in vivo*, *ex vivo* or *in vitro* model systems. Cellular and molecular level effects were included. Study quality (e.g., methodology, group size, unit used for statistical evaluation (litter or fetus), overall reporting) was also considered. Rodent and rabbit studies of pre-conceptional or prenatal oral exposure to Δ^9-THC reported: increased fetal, perinatal, or postnatal offspring mortality (eight experiments); decreased fetal or birth weights (seven experiments); and altered hormone levels or fertility in F1 male offspring (six experiments). Similarly, in studies by parenteral routes of exposure, decreases in fetal or birth weights, and increases in fetal, perinatal, or postnatal offspring mortality were reported. Following postnatal exposure to Δ^9-THC, decreased femoral length in female pups was observed; and following exposure of mouse primary chondrocyte cultures, inhibition of chondrocyte differentiation was reported. Decreased thymic cellularity and increased apoptosis that lasted for several days were observed following prenatal Δ^9-THC exposure. Decreased T cell function in prenatally exposed mice one week after birth has also been reported. The possible role of Δ^9-THC to act via the endocannabinoid system to cause some of the observed effects is discussed.

1614 *In Utero* Exposures to Trichloroethylene and the Development of Congenital Heart Defects: A Systematic Evaluation of the Mechanistic Data

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The hypothesis that *in utero* exposures to low levels of trichloroethylene (TCE) may increase the risk of congenital heart defects (CHDs) in offspring continues to be a subject of substantial controversy within the scientific community, as the principal supporting evidence is a single, irreproducible experimental study in rats. Our previous systematic evaluation of this hypothesis focused on assessment of the risk of bias for epidemiological and experimental animal evidence streams. The objective of the current study was to conduct a systematic evaluation of mechanistic data related to *in utero* exposures to TCE and the development of CHDs. The current TCE-CHD mechanistic evidence base consists of 22 published studies, collectively comprised of more than 70 individual experimental datasets. The datasets were very heterogenous, characterizing endpoints which ranged from molecular to organismal responses in seven species, involving both *in vivo* and *in vitro* study designs in mammalian and non-mammalian models. Of these, 24 datasets were considered reliable following critical appraisal using a study quality tool that employs metrics specific to the study type. Subsequent data synthesis and integration of the mechanistic evidence led to the following conclusions: 1) the database does not support an association between TCE exposure and CHDs, 2) the database does not support the biological plausibility of a response based on assessment of a putative adverse outcome pathway for valvulo-septal cardiac defects, and 3) no datasets were found suitable to serve as candidate studies in risk assessment. Findings supportive of an association were generally limited to *in ovo* chicken studies, in which TCE was administered in high concentrations via direct injection. Results of these *in ovo* studies were difficult to interpret for human health risk assessment given the absence of avian *in vivo* studies and a lack of generalizability of the study models (including dose relevance, species-specific biological differences, variations in the construct of the study design, etc.). Integration of the mechanistic data with findings from previous evaluations of human and animal evidence streams further demonstrates a lack of association between *in utero* exposure to TCE and CHDs in humans.

1615 Linking Lipid and Cholesterol Dysregulation to Cardiac Development in Phenanthrene-Exposed Zebrafish Embryos

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In 2010, the *Deepwater Horizon* (DWH) oil rig explosion released over three million barrels of crude oil into the Gulf of Mexico. The timing of the spill coincided with the spawning of many marine species in the region including mahi-mahi. Previously, RNA-sequencing and mRNA analysis in oil-exposed mahi found significant increases in the abundance of transcripts along the cholesterol biosynthetic pathway. Cholesterol is essential for cardiac development and the disruption of cholesterol and lipid pathways through exposure to polycyclic aromatic hydrocarbons (PAHs) may contribute to the well described cardiotoxicity phenotype in developing fish embryos. To better characterize mechanisms underlying oil-induced lipid and cholesterol declines, zebrafish embryos at 6 hours post-fertilization (hpf) were exposed until 72 hpf to three concentrations of phenanthrene (a reference PAH found in oil), with subsequent qPCR analyses of several genes involved in cholesterol synthesis, as well as staining for cholesterol and neutral lipids. Fluorescence microscopy revealed a significant decrease in antibody-bound cholesterol (14.2% ± 0.6) in the larval body with an even greater reduction in the heart (43.6% ± 1.9). Oil Red O staining for neutral lipids demonstrated decreased staining in the larval head and trunk (5.7% ± 0.5). These reductions corresponded with pericardial edema and bradycardia, as well as increased yolk size. Additionally, when embryos were treated from 6 - 24 hpf with 10μM water-soluble cholesterol, followed by 20-25μM phenanthrene from 24-48 hpf, heart rate increased by an average of 10.7% ± 0.7 across all cholesterol pretreated groups, indicating that cholesterol has a protective effect against bradycardia though the rescue is not specific to phenanthrene treatment. Studies are currently underway to address whether the reduction in cholesterol observed at 72hpf is a result of the maldeveloped heart, which then impairs yolk utilization, or if changes in cholesterol may be impacting downstream development, including heart formation. *This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER).*

1616 Intratracheal Administration in Juvenile Sprague Dawley Rats

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Data generated from juvenile toxicity studies can contribute to the assessment of potential drug toxicity in the pediatric population that are not adequately assessed in reproductive toxicity risk assessments and standard toxicology studies. Direct intratracheal administration is a highly effective pulmonary delivery route bypassing first-pass metabolism and permitting a direct and local delivery of a small amount of drug to the lungs. The objective was to establish a single and repeat intratracheal administration of saline in juvenile Sprague-Dawley rats at 4, 10 and 14 days of age. Prior to intratracheal administration of saline, each juvenile rat was anesthetized by inhalation of an isoflurane / oxygen mixture. The tracheal opening was located in rats using the transillumination method. Saline (≤1 mL/kg) was delivered using a Penncentury® Microsprayer or a feeding needle (24G X 1cm). To confirm the intratracheal dosing procedure, some rats were dosed with Evan's blue dye mixed in saline and determined in lungs at necropsy. Rats were dosed once or daily with saline for 5 consecutive days. Mortality, clinical signs and body weight were monitored after dosing. Rats were euthanized upon completion of dosing and blood was collected via cardiac puncture. Lung weight was measured and bronchoalveolar lavage was collected. Rats survived a single and repeated dose of saline using the trans-illumination method with a Penncentury® Microsprayer or feeding needle administration with minimal clinical signs. In addition, dose volume, bodyweight, blood and bronchoalveolar volumes will be used as a reference for future juvenile toxicology studies.

1617 Hematology and Biochemistry Reference Data in Juvenile Rats at Four, Seven, and 21 Days of Age

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Due to physiologic differences between neonates, children and adults, juvenile animal models of relevant age must be used as test systems in non-clinical studies, in order to evaluate the safety of drugs targeting pediatric human patients. Inherent differences exist between mature and immature biolog-

ical systems. As such, certain markers of toxicity, like the clinical pathology parameters of juvenile animals differ from those of their adult counterparts. Therefore, for the interpretation of results observed in juvenile toxicology studies, age-specific sets of standard clinical pathology parameters are needed for reference. In order to obtain reference hematology and clinical chemistry data from naive Sprague Dawley rat pups, our laboratory collected, processed and analyzed samples from a total of 391 male and female animals at Post Natal Days (PND) 4, 7, and 21. The standard hematology and clinical chemistry parameters used in general toxicology studies were measured from rat pups at each of the critical stage mentioned above. Each parameter was statistically analyzed and compared to the datasets available from adult Sprague Dawley rats. The data analyses showed important differences in the early stages of development when compared to data obtained from adult rats. In addition, the clinical pathology data obtained from rat pups at different stages of their development can be used as a reference ranges for juvenile toxicology studies.

1618 Drug Administration in Neonatal and Juvenile Rats: Challenges and Opportunities

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As regulatory agencies now require that the safety of drugs aimed at treating medical conditions affecting pediatric patients be assessed in juvenile animals, there is an increasing need for neonatal and juvenile animal models of relevant age to be used as test systems in non-clinical studies. The administration of test materials to neonatal and juvenile rats presents several challenges including, the size of the animal, its fragility and the lack of acclimation to the procedures, such as dosing and handling. Our laboratory developed techniques to administer dose formulations intravenously, intramuscularly or subcutaneously to rats as young as two days of age, and as often as twice daily, and by oral gavage to rats as young as four days of age. Given the small size and the fragility of the rats at such a young age, animals are identified using a unique tattoo system that employs indelible digit ink. For dosing, animal movement is usually restrained using wet ice, which also serves as anesthetic. Animal handling prior to and during dose administration, as well as the animals' recovery following dosing are critical to the success of the techniques. Another major challenge is data interpretation, since the biological systems of neonatal, juvenile and adult populations are different. We compiled a database for clinical pathology parameters from naïve rat pups at 4, 7, 14, and 21 days of age. Such database will greatly help interpretation data obtained from pups of different ages.

1619 Effects of Toxicological Test Process on Juvenile Animal Development

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In medical field, the need for safety testing of drugs treating children has been continuously demanded. Adults and children may have different tolerance or mechanisms of toxicity due to their differences in drug sensitivity. So importance of guidelines requiring safety and efficacy of drug for children is emerging because of about 70% of drugs currently on the market are marketed based on non-clinical and clinical trial results from mature individuals. In line with these trends, we continued to conduct toxicity tests on juvenile animals and investigated the impact of these toxicological treatments on juvenile animals. The effect of toxicological treatment was confirmed by using 10-12 weeks old rats which one group of rats that received oral administration of injection water for 8 weeks from 3 weeks after birth and other group of rats that received water orally for 4-6 weeks from 6 weeks after birth. As a result, we found a significant increase of weight gain in group of rats that were treated for 8weeks from 3 weeks after birth. It has been confirmed that the overall health effect of toxicological treatment is insignificant except in weight gain by comparing the relative weights of other organs, (brain, heart, kidney, liver, etc.) other parameters shown slight changes which were within the normal range. These results showed that our toxicity test process for juvenile animals could confirm stable and consistent results in toxicity test.

1620 Use of Extended One-Generation Reproductive Toxicology Studies in Safety Assessments under REACH Regulations

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The safety of chemicals imported into or produced in Europe must be evaluated according to volume, within the scope of REACH regulations. Part of the testing strategy is covered by OECD test guideline 443, which describes the objectives and procedures of the Extended One-Generation Reproductive Toxicity Study (EOGRTS) in rats to be performed on chemicals with volumes over 100 tons if effects are observed in prenatal testing (OECD 414). We have validated methods for the evaluation of EOGRTS endpoints in the parental (P) generation and in three cohorts of their offspring (F1 generation): Cohort 1 to assess reproductive and developmental endpoints and to monitor reproductive performance through mating and parturition; Cohort 2 to assess the impact of chemical exposure on the developing nervous system using neurobehavioral evaluations and brain morphometry measurements; and Cohort 3 to assess potential effects on immune function. In P and cohort 1a and 1b groups, data were collected during evaluations of vehicle treated animals providing normal reference values for P and F1 reproductive and developmental endpoints. For cohort 2, a validation study of the auditory startle apparatus was performed by comparing startle reflex responses in Clonidine treated rats and untreated rats. Decreased amplitudes and increased latency in these responses were detected in clonidine treated rats, demonstrating reliability and sensitivity of the test system. For cohort 3, a reference study was conducted comparing anti-KLH IgM concentrations before and 5 days after KLH immunization. Increased serum concentrations of anti-KLH IgM were observed in animals after immunization, demonstrating a reliable, antigen-specific immune response. In conclusion, these methods show how the EOGRTS study can be used to provide relevant reproductive and developmental, neurological and immune function data for the safety assessment of chemicals under REACH Regulations.

1621 Procedure Development for Repeat Infusions in Juvenile Monkeys

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With the increase of pediatric pharmaceuticals in preclinical studies, the ability to learn and adapt to the use of a younger and smaller test model is necessary to assure process evolution and stress reduction on the research animals. The cynomolgus macaque is a commonly used non-human primate in research. Method development for a repeated-infusion dose in juvenile non-human primates required a united effort to create a concise study and equipment design, with animal welfare at the forefront of all considerations. The routine method for infusion restraint included the use of a procedure cage where all limbs were secured, providing limited movement. This method would be highly stressful for the animals under a year of age needed for the proposed study (juvenile animals 6 to 9 months of age at initiation of dosing). To avoid stress, the "snuggle wrap" method was developed to allow young animals to remain immobile while still feeling comforted by a hugging-like sensation. Stress behaviors were noticeably reduced when using the snuggle wrap compared to manual holding as a restraint method. The snuggle wraps were designed for vein accessibility, while the remaining limbs are secure and comfortable. Given that social pairs needed to remain in proximity, the "snuggle board" was also developed. Animals were implanted with RFID chips, and the wrapped juveniles were labeled with temporary ID cards to allow for easy identification once snuggled and placed on the board. These boards hold 4 snuggled animals closely together which keeps social pairs in visual and auditory contact during dose administration. Following catheter placement, dosing limbs were secured with Velcro straps for the dose duration. Animals were visually monitored, provided treats, juice, light projections, and chewing toys throughout dose duration by trained technicians. The snuggle board restraints were used for ~13 weeks, until the animals were over a year old and large enough for the procedure cage. This method was also successfully implemented in animals as young as 3 months.

1622 Gestational Low-Dose Exposure to Arsenic Leads to Adverse Metabolic Alterations in Adult Mice: A Perspective on Fetal Reprogramming of Metabolism

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Maternal stress or exposure to certain toxicants alter fetal growth and can have permanent effect on offspring, leading to increased predisposition to cardiovascular, metabolic and neuropsychiatric disorders. Maternal exposure to arsenic is a common health menace in many countries but little is known about its effect on disease risk in the exposed progeny. In the current experiment we evaluated the long term effect of only prenatal exposure to arsenic in terms of fetal metabolic reprogramming and its effect on adult onset obesity and dysregulation of glucose metabolism. Pregnant Balb/c mice exposed to very low environmentally relevant concentrations of arsenic (0.4 and 0.04 mg/kg body weight) showed its accumulation in the developing placenta but the levels of arsenic in the embryo were below detection limit. Male Balb/c progeny exposed prenatally to arsenic showed increased body fat mass, hyperinsulinemia, decreased glucose tolerance, insulin resistance, hyperleptinemia and an increase in relative visceral fat content at 40 weeks of age. Cytokine profile of the blood showed an elevated adipokine signature e.g. TNFα, associated with obesity. Dissecting the signaling pathways involved in glucose metabolism revealed activation of JNK1/2 pathway in the visceral fat tissue of the animals. Increased JNK activity has been earlier shown in induced and genetic (ob/ob) mouse models of obesity as well as in obese humans and is also known to regulate adipokine. Based on these observations we conclude that arsenic disrupts fetal programming of metabolism. While the evolutionary purpose of the fetal reprogramming is to optimize the fitness of the offspring for its probable environment, exposure to environmental pollutants such as arsenic during gestation may disrupt this process leading to adult onset health complexities.

1623 The Role of Dietary Zinc in Cadmium-Enhanced Non-Alcoholic Fatty Liver Disease

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Environmental exposure to cadmium (Cd) is implicated in the development of several metabolic syndrome (MetS)-related diseases including non-alcoholic fatty liver disease (NAFLD), however, the ability of early-life, *in utero* Cd exposure to cause obesity-induced MetS in adulthood is poorly understood. Therefore, we developed an *in vivo* two-hit model to study the effect of whole life, low dose Cd exposure and high fat diet (HFD) on MetS with an emphasis on NAFLD. Additionally, we investigated the impact of dietary zinc supplementation and deficiency on disease outcome as both obesity and Cd disrupt zinc homeostasis. Zinc deficiency is common in both obese and NALFD patients. Adult male and female C57BL/6J mice fed normal diets (ND) were exposed to 0, 0.5 or 5 ppm Cd-containing drinking water for 1 week before breeding. Pregnant dams and offspring (F1) were continuously exposed to the same Cd concentration. At weaning, offspring were fed ND or HFD. Male mice were further grouped into diets containing 10, 30, or 90 mg zinc/4057 kcal, representing deficient, normal and supplemented zinc diet, respectively. Body weights and water consumption were recorded weekly. Prior to sacrifice changes in body fat composition were assessed using DEXA scan technology. Insulin resistance was characterized via intraperitoneal glucose tolerance tests, and serum insulin, triglycerides (TG) and cholesterol (TC) levels. Animals were sacrificed 10 or 24 weeks after weaning. Blood samples were collected and liver tissue was snap-frozen, fixed for histopathological analyses or used for metals analysis. Liver to body weight ratios, plasma aminotransferase, and hepatic TG and TC were determined. Thus far we show body weight was impacted by HFD, Cd exposure and altered dietary zinc. HFD altered body composition in males and females whereas Cd exposure altered body composition in males only. Glucose handling was altered in HFD fed males and to a lesser degree in females whereas Cd nor altered dietary zinc impacted glucose handling. HFD caused liver injury in males and Cd exposure exacerbated this injury. Overall, results from this study will provide insight into the mechanisms by which whole life, low dose Cd exposure enhances HFD-induced MetS and discern a potential therapeutic role for zinc in MetS.

1624 Gestational Exposure to Low-Dose $CdCl_2$ Sex-Specifically Induces Hepatic Insulin Insensitivity and Metabolic Syndrome in Adult Female CD-1 Mice

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Obesity and type 2 diabetes (T2D) incidence is rapidly rising and is now a global pandemic that affects more than 400 million individuals. Exposure to environmental contaminants contribute to the pathogenesis of metabolic syndrome, a precursor for T2D characterized by increased body fat and circulating triglycerides, impaired insulin sensitivity and secretion, and increased blood pressure. Cadmium (Cd) is a global contaminant that is increasing in the environment due to human activities including use of fossil fuels and waste disposal. Epidemiological studies have associated prenatal Cd exposure and adult metabolic disease with some female-specific metabolic effects. In animal studies, prenatal Cd exposure at concentrations ranging from 1 - 50 ppm altered liver epigenetics and caused female-specific changes in carbohydrate and lipid metabolism. To explore the obesogenic potential of gestational Cd exposure at concentrations observed in vulnerable populations, CD-1 mice were exposed to 500 ppb $CdCl_2$ via drinking water during gestation and through postnatal day (PND) 10. The presented results demonstrate that gestational low-dose Cd exposure alters hepatic insulin signaling, induces nonalcoholic fatty liver disease (NAFLD), and causes obesity in adult female, but not male, offspring. Cadmium-exposed offspring had increased birth weight in both sexes that normalized by PND7. By adulthood, exposed females were 30% heavier and had a greater than 7-fold increase in perigonadal fat pads. Gestational Cd exposure also decreased insulin responsiveness, increased circulating triglycerides, and increased hepatic lipid accumulation in female offspring. RNA-sequencing of the hepatic transcriptome at PND42 revealed striking differences in females with over 5000 genes differentially expressed. Gene ontology and network analysis identified key pathways including hepatic insulin signaling, NAFLD, and fatty acid metabolism. These findings demonstrate that gestational Cd exposure in mice, at concentrations to which many pregnant women are exposed, induces metabolic syndrome in female offspring. Further analysis of sexually dimorphic responses to gestational Cd exposure and the ontogeny of Cd-induced changes in the hepatic transcriptome will be important for women's metabolic health.

1625 Interaction of the BCRP Transporter with Cadmium in Human Placentas and Cultured Trophoblasts

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Cadmium (Cd) is a ubiquitous environmental metal that is detectable in 97% of pregnant women. Animal and human studies have demonstrated that *in utero* Cd exposure reduces birth weight and perinatal growth. The developmental toxicity is due in part to the accumulation of Cd in the placenta which induces cellular stress, disrupts hormone production, and limits nutrients transfer to offspring. One mechanism to limit Cd accumulation in the placenta is through regulation of uptake and efflux transporters. The BCRP/ABCG2 efflux transporter is highly expressed on syncytiotrophoblasts in the placenta, however, its ability to remove Cd and protect the fetus against toxicity is unknown. In the current study, we sought to 1) assess relationships between Cd concentrations and transporter expression in healthy, term human placentas (N=28) and 2) investigate responses to Cd in cultured human BeWo trophoblasts with reduced BCRP expression. In human placentas, there was a 10-fold range in Cd (as measured by ICP/MS) with a median Cd concentration of 2.9 ng/g. Placentas with Cd > 2.9 ng/g (N=14) had higher mRNA expression of BCRP (*p=0.04),* as well as the Cd uptake transporters DMT1 (*p=0.07*) and ZIP8 (*p=0.04*) compared to placentas with Cd < 2.9 ng/g (N=14). Birthweights were also lower among offspring with placental Cd>2.9 ng/g (mean difference=285 g, *p=0.06*). Knockdown of BCRP expression and function in human BeWo trophoblasts using shRNA heightened susceptibility to Cd cytotoxicity as assessed by alamarBlue and propidium iodide staining. Taken together, these data indicate a dynamic relationship between the placental BCRP transporter and Cd accumulation in the placenta, which may impact the sensitivity of the fetus to Cd-induced developmental toxicity. *Supported by R01ES029275 and P30ES005022.*

1626 Ozone Exposure during Implantation Impairs Offspring Metabolic Processes in a Sex-Specific Manner in Rats

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Intrauterine growth restriction (IUGR), attributable to adverse prenatal environments, is implicated in the increasing prevalence of cardiometabolic disease. We have shown that peri-implantation ozone exposure impairs fetal weight similar to IUGR. Accordingly, this study aimed to determine if ozone-induced fetal growth restriction increases susceptibility to postnatal metabolic disease. Pregnant Long-Evans rats were exposed to 0.8 ppm ozone or filtered air during gestation days 5 and 6 (4 hr/d). Then, at postnatal day 45 subsets of male and female offspring were challenged with a 3-day high fat diet (HFD). In offspring from ozone-exposed dams, HFD challenge increased caloric intake and reduced energy expenditure. However, regardless of diet, females from ozone-exposed dams had reduced hepatic expression of lipolytic genes (*Cpt1*, *Cpt2*, *Ppara*) and bile acid regulatory genes (*Fxr*, *Fgfr4*, *Hnf4a*, *Bsep*, *Ntcp*). Furthermore, postnatal HFD challenge of female offspring from ozone-exposed dams altered hepatic gene expression related to insulin signaling (*Insr*, *Irs1*, *Irs2*), inflammation (*Tnfa*), gluconeogenesis (*Fbp1*, *G6pc*), cholesterol synthesis (*Mvd*), and bile acid signaling (*Cyp27a1*, *Cyp8b1*, *Cyp7b1*, *3βHSD3*, *βKlotho*). Female offspring from ozone-exposed dams also had reduced hepatic triglycerides and circulating insulin levels relative to controls. In male offspring from ozone-exposed dams, alterations in gene expression were mainly attributable to HFD challenge - affecting lipolysis (*Cpt1*, *Cpt2*, *Ppara*) and cholesterol synthesis (*Hmgcs1*, *Mvd1*), but not bile acid signaling. These male offspring also had reduced hepatic triglycerides, increased circulating cholesterol, and increased body adiposity. Together, these findings demonstrate a sex-specific predisposition to metabolic alterations in terms of hepatic responses to pre- and post-natal stressors, highlighting the importance of sex in risk assessment of hepatoxicity and metabolism. This study provides insight on the effects of acute gestational ozone exposure on systemic metabolic status in offspring, which may increase susceptibility to metabolic diseases when challenged with environmental stressors. *Does not reflect US EPA policy.*

1627 Exposure to Nanomaterials during Gestation Affects Cardiovascular Health for at Least 12 Months

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Nanosized - titanium dioxide ($nTiO_2$), is a naturally occurring oxide of titanium and is intentionally manufactured for use in a wide range of applications including industrial and personal care products. In laboratory studies, $nTiO_2$ has been used as a surrogate for ultrafine air pollution particulate. We have shown that exposure to $nTiO_2$ during pregnancy affects the cardiovascular health of the dam, fetus, and young adult offspring. It is unclear whether cardiovascular dysfunction in the progeny persists into middle age. Timed-pregnant Sprague-Dawley rats (Charles River) were exposed to $nTiO_2$ aerosols [9.65 ± 0.07 mg/m^3, primary particle size 21 nm, agglomerate size 128.45 ± 1.83 nm (SMPS, TSI), calculated daily deposition 47.27 ± 1.88 µg] for 4 hours over 6.00 ± 1.31 days of the remaining gestation via inhalation (HPGA, IESTechno). A subset of animals was exposed to filtered air as controls. Animals delivered in-house, offspring were monitored weekly, and were sacrificed at 12 months. Pressure myography (LSI, Burlington, VT) was conducted to evaluate microvascular reactivity in coronary arterioles to assess endothelium-dependent (EDR) [acetylcholine (10^{-9} - 10^{-4} M)]. Gestational $nTiO_2$ exposure significantly impaired EDR relaxation (43.80 ± 5.49 %) compared with controls, a finding maintained from previous studies of young adult offspring. Following arteriolar isolation, hearts were collected, fixed in 10% formalin, sectioned, and reviewed for histopathological analysis. Samples from exposed offspring present histopathological alterations characterized by multifocal myocardial inflammation, degeneration, necrosis, loss and/or fibrosis compared with control tissue. These findings were consistent with repeated low-grade ischemia. Anecdotally, a neurological tick and seizure activity were observed in 5 female animals exposed *in utero* to $nTiO_2$. Brains from these animals were collected, fixed for 24 hours, sectioned, and reviewed. Early observations indicate histopathological changes within the pituitary. Overall, gestational exposure to $nTiO_2$ produced coronary microvascular dysfunction, accompanied by histological changes in the heart that persist to middle age. These results may provide experimental evidence for a better understanding of the developmental onset of cardiovascular disease after gestational exposure to aerosolized xenobiotic particulates. *Supported by NIH-R00-ES024783; P30-ES005022; T32-ES007148.*

1628 Male-Specific Upregulation of Insulin-Like Peptides following Preconception Exposure to Perfluorooctanesulfonic Acid (PFOS) in *Drosophila melanogaster*

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The mechanisms by which preconception toxicant exposure contributes to metabolic dysfunction in later life are poorly understood. Perfluorooctanesulfonate (PFOS) is an industrial toxicant that has infiltrated water and air systems. Due to the long half-life, bioaccumulation potential, and near omnipresence in human urine samples tested, it is important to gain an understanding of the mechanism of PFOS activity and its impact on human health. Preliminary work from our collaborators in zebrafish embryos has shown that preconception exposure to PFOS alters pancreatic development and the expression of pancreatic hormones essential for metabolic regulation. Based on this work, we used the *Drosophila melanogaster* model system to test the hypothesis that preconception PFOS exposure results in dysregulation of insulin hormone expression in progeny throughout their lifespan. Virgin female animals were dosed with 2ng of PFOS 24 hours prior to mating and progeny were collected at eclosion (1 day), peak reproduction (5 day) and old age (14 day). Preliminary results using qPCR show upregulation of *Drosophila* insulin-like peptides 2, 3 and 6 at eclosion, showing a 6 fold, 6.5 fold and 2.5 fold increase in expression respectively in male progeny while female progeny are indistinguishable from vehicle controls, n=10 individuals per experimental group. This data suggests that preconception PFOS exposure has a sex-dependent effect on metabolic regulation. This study will provide insights into how exposure to environmental toxins are contributing to the increased prevalence of metabolic dysfunction and further studies aim to understand the persistence of this effect throughout the lifespan of the animal. *This work is funded by grant R01ES028201 from the National Institute of Environmental Health Sciences, National Institutes of Health.*

1629 Preconception Exposure to Perfluorooctanesulfonic Acid (PFOS) Alters Markers of Metabolic Disease in *Drosophila melanogaster*

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Preconception factors such as nutrition and chemical exposures influence oocyte developmental processes, potentially resulting in adverse health outcomes for offspring. Though the role of preconception nutrition is established, the effects of environmental toxicants on oocyte maturation and offspring health have been understudied. Perfluorinated compounds (PFCs) are surfactants used in non-stick surfaces that have infiltrated the environment, with related compounds detected in 98% of human urine samples. PFC exposure is linked to an increased risk of metabolic syndrome, though the mechanism by which preconception exposure to PFCs alters oocyte maturation and adult health are not well understood. We used the *Drosophila melanogaster* model system to test the hypothesis that preconception exposure to the PFC family member perfluorooctanesulfonic acid (PFOS) alters oocyte development and markers of metabolic disease such as levels of triglyceride, cholesterol and glucose throughout development. Virgin females were dosed with 2ng PFOS in acetone or acetone control 24 hours prior to mating, progeny were separated into an early group, days 1-2 post mating, and a late group, days 2-4 post mating. Our results show that preconception PFOS exposure results in significant developmental delay in the early group, with the progeny of PFOS exposed animals taking 8.4 days to reach adulthood at 25^{0}C (n=30) compared to 7 days for age matched controls (n=22). Additionally, the early group PFOS progeny showed a 30% reduction in adult body weight compared to controls. The progeny of PFOS exposed mothers also show changes in markers of metabolic disease, with female progeny showing elevated cholesterol levels at 1 and 5 days post eclosion and all progeny showing reduced cholesterol at 14 days (n=10-30 animals/treatment). Triglyceride levels were significantly increased in all progeny at eclosion but were not significantly different from controls at later timepoints (n=20-50/treatment). These results suggest that preconception PFOS exposure is sufficient to alter markers of metabolic disease and acts as a first step towards the ultimate goal of understanding how preconception toxicant exposure predispose individuals to later-life metabolic syndrome. *This work is funded by grant R01ES028201 from the NIH-NIEHS.*

1630 Pancreatic Nrf2 Expression and Organ Morphogenesis Is Altered by Modulating Glutathione in the Developing Zebrafish (*Danio rerio*) Embryo

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Pancreatic islet growth during development is sensitive to redox-active chemicals, but mechanisms governing this are unknown. Building on differential glutathione (GSH; predominant cellular redox buffer) redox conditions during embryogenesis, we used zebrafish (*Danio rerio*) and small molecules to investigate roles of GSH and the transcription factor Nrf2a (Nfe2l2a; zebrafish Nrf2 co-ortholog) in islet morphogenesis. We visualized GSH utilization *in vivo* using monochlorobimane staining, and Nrf2a expression using whole-mount immunohistochemistry at 96 hours post fertilization (hpf), when the pancreas has formed. Chemical GSH modulation at 24 hpf (low GSH levels, reducing redox potentials), soon after pancreatic β cells form, did not induce islet morphology changes through 96 hpf. However, GSH modulation at 48 hpf (low GSH levels, oxidizing redox potentials) induced islet morphology changes persistent through 96 hpf. Decreased β cell cluster area at 96 hpf resulted from prooxidant exposures to *tert*-Butylhydroperoxide (tBOOH; 77.6 μM; 10 minutes at 48 hpf) and tBHQ (1 μM; 48-56 hpf). Conversely, exposures at this stage to antioxidants N-AcetylCysteine (NAC; 100 μM; 48-72 hpf), which bolsters cellular GSH, and SFN (20 μM; 48-72 hpf) significantly increased islet areas (n>30 fish). Nrf2a was also stabilized in the islet at 48 hpf: 10 minute high (776 μM) and low (77.6 μM) tBOOH exposures significantly increased Nrf2a protein in islets, compared to control islets that lack Nrf2a. Next, embryos were chronically exposed to 3.2 or 32 μM PFOS, an environmental toxicant. 3.2 μM PFOS increased GSH levels by 50% in GI tract at 48 and 72 hpf, 32 μM PFOS caused a 60% decrease (n>30 fish). Islets expressed significantly higher Nrf2a at both doses, rescued by concurrent100 μM NAC exposures. To assess functional implications of Nrf2a changes, we used biotinylated cell permeable GSH to visualize *in situ* protein glutathionylation. Islets had high protein glutathionylation, indicating oxidized GSH pools, congruent with low Nrf2a in the islet. The 10 minute low (77.6 μM) tBOOH exposure (induced Nrf2a in islet) decreased islet protein glutathionylation, while the 10 minute high (776 μM) tBOOH exposure (induced Nrf2a in entire pancreas) decreased global protein glutathionylation (n>15 fish). Notably, mutant fish expressing inactive Nrf2a were protected against abnormal islet morphology and aberrant Nrf2a protein expression. Our data show disrupted GSH homeostasis underlies changes in Nrf2a expression. This affects cellular GSH pools and alters protein glutathionylation, disrupting pancreatic β-cell development; significant because many chemicals impact GSH and Nrf2.

1631 Morphine Sulfate Induces Genomic Hypomethylation and Concurrently Downregulates Methyl Transferase Gene Expression in Mouse Embryonic Stem Cells

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Opioid-induced perturbations in the epigenetic (EG) landscape are currently emerging as valuable biomarkers for opioid toxicity. We have previously shown that continuous exposure to morphine sulfate (MS) causes cumulative cytotoxicity and genomic DNA hypomethylation, in mouse embryonic stem (mES) cells. In this study, we hypothesize that MS induced suppression in DNA methylation is associated with concurrent down-regulation in gene expression of DNA mehtyltransferases (DNMTs) in mES cells. To determine the mechanism whereby opioids influence genomic methylation patterns, mES cells were exposed to10 μM MS for 1 to 7 days. Global DNA methylation and gene expression of DNMT1, -3a, and -3b were measured on 1-, 3-, 5-, and 7-days. The results show a constant decrease in genomic DNA hypomethylation on all days measured. Gene expression of DNMT1 were down-regulated on 1-, 3-, and 5-days, whereas gene expression of DNMT3a and -3b were down-regulated on 1- and 3- days, followed by up-regulation on day 5. To determine if these changes were reversible, cells were allowed to recover for 3 days in the absence of MS after each exposure period. Genomic methylation and gene expression analysis after the recovery period revealed a reversal of both MS-induced hypomethylation and down-regulations in gene expression of DNMTs, respectively. DNMT1 is usually referred to as the "maintenance methyltransferase" since it has a higher preference for hemi-methylated DNA. DNMT3a and DNMT3b are the predominant *de novo* MT's. Mammalian embryonic organogenesis is characterized by significant DNA methylation followed by selective demethylation. Consequently, the data suggest that MS induces EG toxicity by redirecting extensively pre-programmed genomic DNA methylation and expression of DNMTs that are normally inherently linked, thereby interfering with organogenesis during embryonic development.

1632 Developmental Exposure to Cannabidiol and Later-Life Consequences

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The use of cannabidiol (CBD) is rapidly increasing due to its indications for many ailments including arthritis, insomnia, anxiety, and pregnancy-associated nausea. However, there is limited knowledge of potential adverse outcomes due to CBD exposure during critical periods of development and the potential long-term effects. Using the zebrafish (*Danio rerio*) model, our goal was to determine the effects of developmental exposure to CBD on later life consequences in the exposed generation as well as subsequent F1 and F2 generations. In the F0, but not F1 or F2 generations, exposure to ≥ 2 μM CBD during development (6-96hpf) resulted in significant larval deformities and mortality. In addition, we observed significant reduction in fecundity in exposed F0 as adults (0.5 μM CBD exposure) compared to unexposed controls, but fecundity in F1 adults was not different from controls. When F0 fish reached 2.5 years of age, they were considered "aged" and CBD effects were assessed by comparing to both aged control and young (4-7 month old) unexposed groups. In the aged fish, developmental exposure to CBD significantly reduced sperm counts, and there was a reduction in size (wet weight and length) of female fish compared to aged controls. Our results support the need to consider the developmental origins of health and disease of CBD including later life-stage effects. *Supported by the National Institute on Drug Abuse R21DA044473-01.*

1633 Pre- and Postnatal Developmental and Reproductive Toxicity Study of the AV7909 Anthrax Vaccine in Rats

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AV7909 consists of the Anthrax Vaccine Adsorbed (AVA) and the immunostimulatory Toll-like receptor (TLR) 9 agonist oligodeoxynucleotide adjuvant CPG 7909 and is similar in composition and manufacturing process to the commercial BioThrax® product. The purpose of this study was to evaluate the potential maternal, reproductive, and developmental toxicity of the AV7909 vaccine in rats. F_0 female Sprague Dawley Crl:CD (SD) rats (44/group) were dosed by intramuscular injection in the thigh muscle with water for injection, adjuvant (CPG7909/Alhydrogel in saline) or AV7909 at a volume of 0.5 mL/dose. Animals received three vaccinations: 14 days prior to start of cohabitation, on the first day of cohabitation and on gestation day (GD) 7. Half of the F_0 females in each group were randomly assigned to laparohysterectomy on GD 21 and half were assigned to natural delivery and allowed to rear offspring until postnatal day 21. There was no effect on mating, fertility, pregnancy, embryo-fetal viability, growth, or morphologic development, parturition, maternal care of offspring or postnatal survival, growth or development. There was no adverse maternal toxicity in this study; test article-related findings in F_0 females were limited to non-adverse (transient, predominantly very slight to slight severity) injection site edema and non-adverse (small, transient) injection site nodules visible grossly at necropsy. There was no systemic tissue injury or systemic inflammation in pregnant rats, based on evaluation of serum concentrations of the acute phase proteins alpha-2-macroglobulin and alpha-1-acid glycoprotein two weeks after the last of three administrations of AV7909. As expected, based on the ability of maternally derived antibodies to cross the placenta and transfer via the mother's milk, AV7909 antibodies were detected in the serum of fetuses and pups. High titers of anthrax lethal toxin-neutralizing antibodies were detected in F_0 females and their fetuses at the end of gestation and in F_1 pups at the end of lactation in the AV7909 group. Therefore, exposure of the fetuses and pups to maternally-derived AV7909 antibodies did not result in any developmental toxicity.

1634 Short- and Long-Term Effects of Perinatal Exposure to Phthalates and Phthalate Mixtures on Mouse Liver Metabolism

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Phthalates have been labeled as "obesogens" and interfere with metabolism by interacting with peroxisome proliferator-activated receptors (PPARs). Development is a critical window of exposure for obesogens, including phthalates, but mechanisms linking developmental exposures and long-term metabolic health outcomes are not fully understood. We hypothesized that developmental exposures to two phthalates, diethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP), and a mixture of the two (DEHP+DINP), reprogram PPAR target genes in the liver with long-lasting impacts on lipid metabolism. We used an established isogenic mouse model of perinatal exposures through the diet with an exposure window 2 weeks prior to mating until weaning at postnatal day 21 (PND21). Livers were collected from mice at PND21, at the end of the exposure period, and at 10 months (10M), long after exposure ceased. We utilized transcriptomics followed by pathway enrichment analyses to identify PPAR pathways that were altered at both time points. We found that acetyl-CoA metabolism was enriched in livers from mice perinatally exposed to DINP (FDR=0.0018), an effect that was specific to females. Of significant genes driving this pathway, 10/13 were PPAR target genes, and 9 were upregulated, including *Acly*, *Cs*, and *Fasn.* To characterize functional metabolic liver outcomes, two targeted metabolomics assays were carried out in females: a central metabolism profile and an acylcarnitine profile as a measure of fatty acid oxidation. Relative to controls, females perinatally exposed to DEHP+DINP had increased acetyl-CoA at PND21 (adjusted p=0.0005) but decreased acetyl-CoA at 10M (adjusted p=0.013), while acylcarnitines were increased only at 10M in DEHP, DINP, and DEHP+DINP females (p=0.011, 0.033, and 0.061, respectively). Together, our data indicate that perinatal phthalate exposures were associated with increased mRNA expression of PPAR target genes at both PND21 and 10M, which manifested as increased fatty acid production in early life and increased fatty acid oxidation in adulthood.

1635 The Intergenerational Metabolic and Behavioral Effects of Paternal Phthalate Exposure

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Phthalates in plastics and cosmetics have been reported to play a role in a host of behavioral and metabolic outcomes. However, little is known about intergenerational effects of exposure. Without such knowledge, our understanding of paternal environmental determinants of offspring health is limited. We hypothesized, based on acute exposure data, that paternal phthalate exposure in mice would induce behavioral and metabolic changes in offspring phenotypically similar to ADHD and diabetes in humans. Adult reproductive age male C57B/L mice (F0) were treated with 5.0 mg/kg/day of DEHP, 5.0 mg/kg/day of DBP, or a mixture of the two (5.0 mg/kg/day of DEHP and DBP) for 70 days to cover 2 spermatogenesis cycles. Controls were exposed to the vehicle only. After exposure, F0 male mice were bred with non-exposed females, and one male and one female from each F1 litter were selected for phenotypic testing. Open field and elevated plus maze tests were conducted for all F1 mice, and a three-chamber sociability test was conducted for each F1 male mouse. A glucose tolerance test was performed on F1 male mice and glycosylated hemoglobin was measured. Statistically significant changes in exploratory behavior in the open field test were observed, with an increase in exploratory behavior-time spent in the center of the maze-for mice in the DEHP group relative to controls, with increased significance in males ($p<0.001$). However, this change in behavior related to paternal DEHP exposure was abolished in mice whose fathers were exposed to both DBP and DEHP ($p<0.01$). There were trends toward statistical significance for differences in blood glucose at multiple time points between male mice, and a statistically significant decrease in body weight for male mice paternally exposed to DEHP and DBP relative to controls ($p<0.05$), indicating faster glucose absorption by tissues in exposed animals. However, glycosylated hemoglobin levels were not statistically significant for any group, indicating that long-term levels of glucose may not be affected by paternal phthalate exposure. Therefore, significant changes in behavior and metabolism in offspring of exposed males suggest that paternal phthalate exposure influences offspring behavior and metabolism possibly through a non-genetic mechanism. Our data suggests that paternal phthalate exposure may play a role in offspring health.

1636 Elucidating Epigenetic Mechanisms Involved in the Origins of Adult Metabolic Phenotypes following *In Utero* DEHP Exposure

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Environmental exposure to Endocrine Disrupting Chemicals (EDCs) during fetal development may lead to molecular changes that increase susceptibility to adult diseases. For example, EDCs can alter fetal growth and metabolism *in utero* and disrupt critical set points that promote adult metabolic disorders. The EDC Di-(2-ethylhexyl)-phthalate (DEHP) is strongly associated with metabolic changes. DEHP is a ubiquitous plasticizer, present in food packaging, toys, medical devices, and personal care products. DEHP has anti-androgen functions in fetal/placental endocrine systems, interfering with hormonal action. As a result, DEHP exposure during pregnancy may result in adult metabolic defects. However, the mechanisms driving DEHP-induced changes in fetal metabolism and adult diseases are unknown. Recent evidence suggests that EDC-induced changes in metabolic gene profiles may stem from altered epigenetic landscapes. One epigenetic factor, DNA methylation, is particularly vulnerable to environmental exposures as it changes dynamically during fetal development. Thus, we hypothesize that DEHP exposure *in utero* disrupts DNA methylation in placenta, leading to abnormal cross-talk between the fetus and placenta, which in turn will predispose offspring to impaired metabolism in adulthood. To test this, dams were exposed to two DEHP doses (Lower: 50 µg/kg/BW, Upper: 10 mg/kg/BW) via their diet from pre-conception until embryonic day 10.5 (E10.5) or weaning. Post-weaning, offspring were placed on a control or Western Diet (WD) until postnatal day 182 (PND182). Global DNA methylation was assessed on E10.5 placentas using a Luminometric Methylation Assay. Maternal exposure to upper-DEHP was associated with significantly increased global DNA methylation of E10.5 male placentas. Our data suggest that DEHP alters E10.5 placental global DNA methylation in a dose- and sex-specific manner. Adult metabolic phenotyping included glucose tolerance tests, body weight and body fat. Phenotyping revealed that male offspring exposed to upper-DEHP and challenged with WD have impaired glucose tolerance at PND140 and increased body fat at PND182 while females were unaffected. These results suggest that *in utero* DEHP exposure at environmentally relevant doses disrupts glucose homeostasis in a sex-specific manner. Ultimately this work will evaluate the effects of prenatal DEHP exposure and the mechanisms through which it works to understand human health risks.

1637 The Role of the mTOR Pathway in Developmental Programing of Liver Lipid Metabolism by 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47)

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An emerging hypothesis links the epidemic of metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD) and diabetes with chemical exposures during vulnerable windows of development. Evidence from our lab and others suggests that developmental exposure to environmentally prevalent flame-retardant BDE-47 may permanently reprogram lipid metabolism and results in NAFLD like phenotype. Additionally, we have demonstrated that BDE-47 alters the activity of both mTOR complexes (mTORC1 and 2) in hepatocytes. The mTOR pathway integrates environmental information from many different signaling pathways, and regulates key cellular functions such as lipid metabolism, innate immunity, and ribosome biogenesis. Thus, we hypothesized that the developmental effects of BDE-47 on liver lipid metabolism are mTOR-dependent. We conducted two LoxP-Cre recombinase breeding schemes to generate liver-specific knockouts of *Rptor* (a key component of mTORC1) and *Rictor* (a key component of mTORC2), as well as their respective wildtypes. Mice from all four groups were exposed perinatally to BDE-47, or to vehicle only. Liver and serum were collected from exposed offspring on PND65. Serum triglycerides were analyzed using the Cayman COX Kit, and transcriptomic analysis of hepatocytes was conducted via Illumina RNA-seq. Sequencing data was analyzed using Metascape Ontology and Gene Set Enrichment Analysis. Western blots were also conducted to analyze mTORC1 and 2 activity. Results suggest that developmental exposure to BDE-47 permanently reprograms liver lipid metabolism, innate immunity and other key cellular functions. The transcriptomic effects of BDE-47 appear to be synergistic with suppression of mTORC1, but not mTORC2 activity. Our liver-specific mTORC2 knockout abolished exposure-induced changes in blood triglycerides, while effects of exposure on innate immunity genes are primarily dependent on mTORC1. Differences in transcriptomic responses to BDE-47 exposure between wild type animals from the mTORC1 and mTORC2 breeding schemes suggest that different genetic backgrounds intersect with developmental exposures to determine the lifelong effects of exposure on the liver transcriptome. Overall, our results provide a hypothetical model of

gene-environment interaction in life-long programing of liver lipid metabolism and other functions. Our model suggests that modulation of the mTOR pathway may affect liver susceptibility to environmental exposures.

1638 *In Utero* and Lactational Dioxin Increases Noradrenergic Axon Density and Nerve-Evoked Smooth Muscle Contraction in Mouse Prostate

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In utero and lactational (IUL) TCDD exposure causes mouse prostatic smooth muscle thickening and urinary dysfunction in adulthood consistent with prostate-related urinary symptoms experienced by most aging men. Increased prostate smooth muscle tone and hypertrophy of prostatic smooth muscle are hypothesized to contribute to urinary dysfunction because treatment with alpha-one adrenergic antagonists that relax prostate smooth muscle relieves urinary symptoms. We hypothesize IUL TCDD exposure increases neurotrophin expression in the fetal prostate, thereby recruiting noradrenergic axons and permanently increasing their density, sensitizing prostatic smooth muscle to nerve-evoked contraction and resulting in hypertrophy. Methods: Pregnant dams (control and Myh11-GCaMP5g mice on a C57Bl6/J background) were dosed with TCDD or corn oil (vehicle) at embryonic day 13 ((e)13.5). Control prostates were collected at e17.5, postnatal day (P)9 and P50. Immunostaining was used to quantify noradrenergic axon density. Myh11-GCaMP5g prostates, harboring genetically encoded calcium sensors that fluoresce during muscle contraction, were collected at 14 weeks and stimulated with graded frequencies (1 Hz- 30 Hz) to evoke action potentials and neurotransmitter release from noradrenergic axons. Fluorescent calcium transients were quantified as indices of muscle contraction. Results: IUL TCDD exposure increases noradrenergic axon density in mouse prostate as early as P9 and continuing until early adulthood. IUL TCDD exposed prostatic smooth muscle contracted at a lower frequency than vehicle-dosed prostatic smooth muscle. IUL TCDD exposure permanently increases noradrenergic axon density, increases the sensitivity of prostate smooth muscle to contraction, and thickens prostate smooth muscle. We propose that these TCDD responses derive from an increase in neurotrophins during fetal development, which we are actively investigating. This is the first evidence developmental exposure to TCDD permanently and functionally changes prostate peripheral nerve density. *NIH grants: R01ES001332, K99 ES029537, and T32 ES007015.*

1639 Developmental Reprogramming of the Gut Microbiome and Microbial Metabolites by Early-Life Exposure to Persistent Environmental Contaminants

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Gut microbiome is increasingly recognized as a pivotal player in toxicological responses, and dysbiosis may worsen chemical-induced adverse outcomes such as inflammation, metabolic syndrome, and cancer. Early life exposure to environmental contaminants may produce long-lasting toxicities in adulthood. Little is known to what extent early life exposure to environmental toxicants modulate the gut microbiome beyond childhood. Therefore, this study tested the effects of perinatal exposure to 3 human health relevant environmental contaminants (BDE-47, TBBPA, BPS), on the composition and functions of the gut microbiome of perinatally exposed adult male mice. CD-1 mouse dams were orally exposed to vehicle (corn oil, 10ml/kg), BDE-47 (0.2mg/kg), TBBPA (0.2mg/kg), or BPS (0.2mg/kg) once daily from gestational day 8 to postnatal day 21. Feces from male pups were collected at 12-weeks of age (n=14-23/group). Microbial DNA was subjected to 16S rDNA sequencing, and analyzed using QIIME. Lefse was used to determine microbial biomarkers. Microbial functions as well as key taxa that drive functional changes were predicted using PICRUSt and FishTaco, respectively. To validate the functional shifts in gut microbiome, bile acids (BAs) and short chain fatty acids (SCFAs) in fecal samples were analyzed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), respectively. Principle coordinate analysis showed a distinct separation among different exposure groups, especially between BPS and vehicle exposure groups. The BDE-47, and TBBPA exposure groups overlapped slightly, but remained separated from the vehicle cluster. There was a persistent decrease in many taxa of the *Clostridia* class in the *Firmicutes* phylum to all 3 chemicals, whereas many taxa of the *Bacteroidia* class in the *Bacteroidetes* phylum were persistently up-regulated. Each group had distinct biomarkers which was *Clostridiales* for vehicle, *S24-7* for BDE-47, *Rikenellaceae and LactoBacillus* for TBBPA and BPS respectively. FishTaco predicted that BDE-47 increased microbial BA synthesis, and indeed, fecal total, secondary, and unconjugated BAs were all increased in adult pups following early life BDE-47 exposure. Interestingly, TBBPA and BPS also persistently increased these BA species. Regarding SCFAs, BPS down-regulated acetic acid, whereas TBBPA up-regulated propionic acid and succinate. Together these findings suggest early life exposure to these environmental contaminants produces persistent gut dysbiosis in adult male offspring, leading to functional shifts that may play important roles in regulating certain diseases of the host.

1641 Placental Alterations following Prenatal Arsenic Exposure and Relation to Long-Term Lung Immune Effects

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More than 140 million people around the world are exposed to arsenic in drinking water at levels higher than the 10 µg/L guideline from the WHO. Inorganic arsenic is a confirmed carcinogen and associated with myriad health effects including detrimental immune outcomes. It is well established that prenatal arsenic exposure has cell-specific effects on components of the immune system, resulting in reduced immune efficacy, particularly in the lungs. Preliminary data from our prenatal arsenic exposure mouse model indicates that in adult pups, even ten weeks following exposure cessation, cytokine profiles of immune cells from the lung are altered before and after stimulation with LPS and IFNγ. Furthermore, flow cytometry data indicates a reduction in inflammatory response in macrophages from prenatally exposed animals. To better understand mechanisms driving the observed long-term immune deficit, we will examine the placenta and fetal lung tissue. The role of the placenta in mediating long-term immune effects is largely unknown. Using a mouse model of prenatal (including pre-conception) exposure to sodium (meta) arsenite, we are investigating effects on the placenta and fetal lungs through gene expression investigation (via microarray and RT-qPCR) and flow cytometry. Importantly, results from the current study could result in avenues to pursue early intervention strategies to reduce the long-term effects of early life arsenic and other heavy metal exposures.

1641a E-cigarette Exposure during Development Alters Protein Transporter Activity in Neural Pathways That Are Associated with Obesity in Mice

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Electronic cigarettes (E-cigs) are battery-powered devices that usually contain vegetable glycerin and propylene glycol as humectants, as well as nicotine and added flavors. E-cig use is dramatically increasing in popularity, particularly with adolescents and young adults. According to the CDC, more than 1 in 4 high school students and about 1 in 14 middle school students have vaped in the past 30 days in 2018. E-cigarette use increased from 11.7% to 20.8% among high school students and from 3.3% to 4.9% among middle school students from 2017 to 2018. Although cigarette smoking during pregnancy has been shown to increase the risk of childhood obesity, little research has been carried out to determine if the same obesity propensity is conferred to the offspring by maternal vaping while pregnant. Preliminary mouse data by our laboratory suggest such a connection. Neural pathways in the hypothalamus are involved in hormonal and nutritional signaling that coordinate glucose homeostasis and weight gain. We hypothesized that prenatal exposure to e-cig aerosols, with and without nicotine, alters transcriptional and inflammatory activity in known metabolic pathways in the hypothalamus that are associated with obesity. For this study, pregnant C57BL/6 mice were exposed daily throughout gestation (3h/day; 5 d/wk for about 3-wk) and postnatally from PND 4-21 to e-cig aerosols with or without nicotine and employing the same exposure conditions used during the gestational exposure. At 1-mo-of-age, the brain was removed from both male and female offspring. Expression of transporters associated with obesity, including Glucose 1,2,3, GLAST, PPARγ, AMPA, and GFAP were then analyzed in the hypothalamus of offspring exposed early in life to e-cig aerosols (with or without nicotine) or filtered air by Western blot. Results of this study demonstrated a two-fold increase in glucose transporter expression in the no-nicotine (PG/VG alone) group, and a 2.5-fold increase in the PG/VG plus nicotine group compared to control levels. Given the growing epidemics of both vaping and obesity and the lack of study in this particular scientific area, a better understanding of

the link (if any) between these two epidemic waves is critical for protecting the health of two highly-vulnerable groups, children and pregnant women. *Supported by NYU NIEHS P30ES000260-55*

1642 Application of the US EPA TSCA Study Quality Evaluation System: A Case Study of Health Effects from Residence Near Oil and Natural Gas Development

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Under the Toxic Substances Control Act (TSCA), the United States Environmental Protection Agency (US EPA) Office of Pollution Prevention and Toxics (OPPT) has promulgated systematic review methods to allow for more transparent and objective human health chemical risk evaluation assessments. A key element is a robust and standardized method for evaluating the quality of individual studies, which is a critical step when synthesizing the available literature and drawing conclusions on potential health risks. We present a case study of the EPA TSCA study quality evaluation system for epidemiology within a review of the potential human health effects of exposures associated with residence near oil and natural gas (ONG) production sites. Our review indicated that a tiered approach to the evaluation of study quality placed appropriate emphasis on the domains of quality most relevant to the chemical exposure and health effects of interest. For example, exposure characterization was particularly weak in many ONG studies, which limited the overall confidence in the study regardless of other domain scores. We therefore used exposure assessment as the first tier criterion, moving only to other domains if exposure characterization was of medium or high quality. In our analysis, many studies were of a low overall quality, and no studies fulfilled the TSCA criteria required for a high-quality rating. Thus, while there were associations between residential proximity to ONG development and some health effects, exposure misclassification, bias, confounding, and chance could not be confidently excluded as alternate explanations for the study results. Overall, our evaluation underscores the criticality of assessing study quality in risk evaluation. While the TSCA study quality evaluation system is one of the most comprehensive frameworks available, adaptions may be necessary to ensure that it is fit-for-purpose and not unduly burdensome. Effective methods for integrating evidence across lines of inquiry are still needed.

1643 Daily Variation of Air Pollutants Near an Elevated Highway System

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Interstate-81, which is an elevated viaduct, goes right through the heart of the Syracuse, NY, with local businesses and communities located underneath it. The safety limit of the viaduct is ending, and New York State is looking for recommendations to either rebuild the roadway using its current elevated design or lower the highway to ground level to create a community grid. People living in the neighborhood below the Interstate are concerned about an increase in air pollution if the Interstate is lowered, but with no knowledge of the current air quality levels in their community at the highway level, assessing how lowering the highway would impact the neighborhood is not possible. Therefore, we have started an intensive air quality monitoring campaign to measure air pollutant levels simultaneously near the highway and on the ground below it. Models generated by the Department of Transportation suggest that traffic levels will not change if the highway is lowered, and by monitoring air quality at both the highway and ground level, results from our study will offer a glimpse of potential air quality problems that can arise if the Interstate is lowered. To assess the daily variation in traffic-related particulate matter (PM) near this highway, a sampling site adjacent to the roadway was selected. Two high-volume cascade impactors were placed at both ground and roof level of a building to collect daily fine PM for two weeks over two seasons - summer and fall. Additionally, two real-time PM monitors were placed at each location and measured PM number concentration. For fine PM, the number of particles was, on average, 1.2 times higher, and at maximum, 10 times higher, at the rooftop location. In the summer, three days were noted to have exceptionally high vehicle flow and had fine PM concentrations ranging from 4.5 to 10 times higher at the rooftop level. Results from the gravimetric analysis were in agreement with the measured number concentrations. This data shows that particle count and fine PM concentration are variable with elevation and suggests that vehicle emissions may be more influential at the rooftop location where fine PM was consistently higher than at the ground level. This implies that the air quality may be affected if the community grid option is pursued.

1644 Big Data to Knowledge Analytics Reveals the Zika Virus Epidemic as Only One of Multiple Factors Contributing to a Year-over-Year 28-Fold Increase in Microcephaly Incidence

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During the 2015-2016 epidemic of Zika Virus (ZIKV) in Latin America the geographical distributions of ZIKV infection and microcephaly outbreaks did not match. This raised doubts about the virus as the single cause of the microcephaly outbreak and led to research hypotheses of alternative explanatory factors, such as agrochemical use, vaccinations, and social conditions. We accomplished our main goal of discerning how variables and factors from the built, natural, physical, and social environments simultaneously shaped this ZIKV-related microcephaly outbreak, by demonstrating significant controlled associations among 382 nonredundant variables ZIKV surveillance and multiple determinants of public health from these environments obtained from 5,565 municipalities in Brazil. We compared the variables and factors directly associated with microcephaly incidence positive to ZIKV and those associated with microcephaly incidence negative to ZIKV, respectively, and mapped these in case and control subnetworks. We mapped a subnetwork of variables and factors associated with low birth weight where birth incidence served as an additional control. Nonsignificant differences in variables/factors were observed and weights of associations between microcephaly incidence positive and negative to ZIKV revealed diagnostic inaccuracies that translated to underestimation of the outbreak size. A detailed analysis of association patterns does not support a finding that vaccinations and use of agrochemicals are factors contributing to microcephaly but does raise further concerns about potential hazards and toxicity resulting from exposures from chemical and non-chemical stressors. In summary**,** comparative network inferential analysis of patterns of variables and factors associated with a Zika virus epidemic in Brazil during 2016 coinciding with a microcephaly outbreak identifies multiple contributing determinants and advances our understanding of the effects of exposures to chemical and non-chemical stressors in the built, natural, physical, and social environment.

1645 Man-Made Vitreous Fibers and Non-Malignant Respiratory Disease: A Systematic Review

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Man-made vitreous fibers (MMVF) are a family of inorganic fibers including insulation wools, glass filaments, and refractory ceramic fibers. Insulation wools, including glass wool (GW) and rock/slag wool (RSW), are commonly used as thermal or acoustical insulation in buildings, vehicles, and appliances. In 2002, the International Agency for Research on Cancer (IARC) classified GW and RSW as *not classifiable as to their carcinogenicity in humans* based on the results of three decades of epidemiological research. While decades of epidemiological research consistently demonstrated that individuals occupationally exposed to these fibers do not experience an elevated risk of respiratory system cancer, less is understood about the potential effects of occupational GW and/or RSW exposure on non-malignant respiratory disease (NMRD). We conducted systematic searches of PubMed and Scopus databases with an aim of identifying all epidemiological studies that evaluated the association between MMVF (specifically, GW and RSW) exposure and NMRD. We identified 280 articles, 53 of which warranted full-text review based on a relevant abstract. Additionally, we reviewed references of all relevant articles identified by our search, as well as studies included in the 2002 IARC Monograph. Ultimately, we found 23 epidemiological studies of occupational MMVF exposure and NMRDs. The mortality studies (n=10) consistently reported null findings [SMRs ranged from 54 (95% CI=33-84) to 119 (95% CI=74-182)]. The 13 non-mortality studies were predominately cross-sectional and evaluated a variety of outcomes including self-reported lower and upper respiratory symptoms, pulmonary function, subclinical disease outcomes assessed by imaging techniques, and clinically diagnosed respiratory diseases. Ultimately, the epidemiological evidence suggests that workers exposed to MMVFs do not experience increased risk

of NMRD mortality compared to the general population. The non-mortality studies reported mixed results, but overall suggest that occupational MMVF exposure is not associated with reduced lung function or pleural plaques. Conversely, multiple cross-sectional studies reported an association between MMVF exposure and chronic bronchitis among participants, but the conclusions that may be drawn from this literature are limited by the tremendous heterogeneity in the design of the non-mortality studies, and the infrequent statistical adjustment for asbestos exposure and smoking habits.

1646 Blood Pressure Control, Glycemic Control, and Dyslipidemia among Healthy Adults in the Cape Coast Metropolis, Ghana

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The World Health Organization recommends the implementation of interventions focused on the early detection of clinical risk factors for cardiovascular disease (CVD) as effective strategies for the control of CVD in low resource settings. However, due to health system resource constraints, surveillance capacity for the identification of high-risk populations for non-communicable diseases, including CVD have been inadequate. The purpose of this study was to describe the prevalence of CVD clinical risk factors among healthy adults residing in the Cape Coast metropolis of Ghana. The clinical risk factors assessed included glycemic control, insulin sensitivity, lipid control and blood pressure. The study participants included 70 healthy adults without a previous diagnosis of CVD from Cape Coast metropolis. Blood samples, blood pressure and anthropometric measurement were obtained for each participant. Serum glycated hemoglobin (HbA1c), insulin, glucose, triglycerides, and cholesterol levels were measured. Results: Approximately four out of ten participants were either overweight or obese. Almost three-quarters of the sample were considered prehypertensive or hypertensive. About three in ten were clinically prediabetic. About a third of the participants had high non-HDL cholesterol levels. Triglyceride concentration levels were found to be high in almost 10 percent of the study sample. Approximately six percent were identified as having metabolic syndrome. A significant proportion of the study participants were identified to be at risk for CVD. There is the need for adaptive and less resource-intensive CVD risk-factor screening interventions to allow for the timely detection and management of CVD risk factors in low-resource settings.

1647 Exposomics of Maternal Plasma in Women at High Risk of Preterm Birth

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Preterm birth (PTB) remains a leading cause of neonatal deaths and perinatal morbidity in the US and worldwide. Early diagnostics and intervention for PTB relies on accurate, comprehensive, and sensitive screening of metabolic marker and environmental risk factor during early pregnancy. In this study, high-resolution global metabolomics was used to probe a prospective cohort of women carrying a singleton, non-anomalous gestation while at high risk for spontaneous PTB. In total, 42 participants (25 cases; 17 control) were enrolled in their mid-trimester pregnancy in 2015-2018 at UNC, based on prior PTB records, cervical length, antepartum hospitalization and so forth. Upon enrollment, blood plasma was collected by venipuncture into an acid-citrose dextrose tube. The samples were extracted, analyzed on liquid chromatography quadrupole-orbitrap mass spectrometry, and processed in XCMS for aligning ion features detected in all samples. Partial least square discriminant analysis identified a distinct group pattern, separating cases and control with 32% for t1, 7% for t2, and 0.846 for Q^2Y. Welch *t*-test revealed 812 features of group difference (fold change>1.2, $p<0.05$), out of which a predominating 570 were higher in PTB than in TB control. A novel cheminformatics pipeline was applied, yielding 131 unique structures related to PTB. Remarkably, metabolome shifts spanned from lipids (e.g. cholesterol, acylcarnitines, lysophospholipids, fatty acids, ceramide), amino acids, purines, glycolytic and tricarboxylic acid cycle intermediates, to sex hormones (e.g. estrone) and aryl hydrogen receptor ligands (e.g. biliverdin). While, a number of xenobiotics were discovered, including food components, microbial metabolites, drug derivatives, environmental pollutants (e.g. PAHs), suggesting a role of dietary intake, lifestyle, and exposures in PTB pathogenesis. Overall, the study characterized important metabolome and exposome shifts in maternal plasma for future research seeking countermeasures for PTB.

1648 Total Dioxin Toxic Equivalency and Sex Are Associated with Biomarkers of Liver Disease in ACHS-II Participants

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Persistent organic pollutants including polychlorinated biphenyls (PCBs) and dioxins can activate the aryl hydrocarbon receptor (AhR) and promote non-alcoholic fatty liver disease (NAFLD). Previously, our group demonstrated high prevalence of suspected NAFLD in Phase I and Phase II participants of the PCB-exposed Anniston Community Health Survey (ACHS) cohort. The purpose of this study is to analyze a subset of ACHS-II participants who demonstrated abnormal CK 18 levels (suspected liver disease), and determine associations between *i*) total dioxin toxic equivalency (TEQ) which represents AhR activation, or *ii*) sex and serological biomarkers of liver disease. Two hundred nine (62%) ACHS II study participants with abnormal CK18 (CK18 M65>300 U/L) were included in the present analyses. Biomarkers (log_e-transformed) of interest were assessed for their joint association with total dioxin TEQ (low/high) and sex (male/female) using a linear model. Results: Participants with high total dioxin TEQ tended to be older (68 y *vs.* 56 y) and were more likely to be female (84% *vs.* 52%), African-American (52% *vs.* 37%), and have a history of diabetes (53% *vs.* 34%). In models without adjustment, high total dioxin TEQ was positively associated with VLDL, TNFα, and hyaluronic acid and negatively associated with albumin. The association with male sex was positively associated with VLDL and albumin. After adjusting *p*-values, associations between high total dioxin TEQ and TNFα (p_{adj}=0.047), and hyaluronic acid (p_{adj}<0.001) remained. In lipid-adjusted models, high total dioxin TEQ was not associated with any biomarker, except albumin. However, persistent sex associations with VLDL and albumin were observed in both unadjusted and adjusted models. Mechanistic biomarkers for lipid metabolism, inflammation, fibrosis and liver function were associated with high total dioxin TEQ, implicating the role of AhR activation by environmental chemicals as a potential mediator in liver disease. The results also suggested that sex plays a crucial role in determining NAFLD outcomes and confirmed our previous findings that dioxin-like chemicals are associated with mechanistic fatty liver disease in ACHS-II participants.

1650 Proximity and Density to Hydraulic Fracturing Wells and Birth Outcomes in Northeastern British Columbia, Canada

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Northeast British Columbia (Canada) is an area of intensive hydraulic fracturing for natural gas exploitation. Concerns have been raised regarding the potential health effects of contaminants emitted by gas exploitation. Some epidemiological studies have found associations between proximity/density of wells and negative birth outcomes. Our objective was to assess associations between proximity/density of wells and birthweight, small for gestational age (SGA), preterm birth and head circumference. We used birth records from the Fort St John hospital from January 1 2007 to December 31 2016 (n = 6333 births). We constructed three exposure metrics by calculating the inverse distance-weighted (IDW) sum of wells within three buffers around maternal postal code centroid: 2.5, 5 and 10 km. IDWs were categorized into quartiles. We then used linear or logistic regression to evaluate associations between IDW quartiles and birth outcomes. Models were adjusted for infant's sex assigned at birth, parity, maternal age and smoking status. We found increased adjusted odds of preterm birth associated with proximity/density of wells in the 2nd (odds ratio [95% confidence interval] = 1.60 [1.30, 2.43]) and 3rd quartiles (1.34 [0.90, 2.08]) of the 2.5-km buffer. We found significant negative associations between birthweight and proximity/density of wells in the 2nd (adjusted beta [95% confidence interval]: -40.9 g [-78.0, -3.7]) and 3rd (-42.0 g [-79.2, -4.9]) quartiles of the 5-km buffer;, and in the 3rd quartile of the 10-km buffer (-47.3 g [-84.3, -10.3]). We found no significant association with SGA or head circumference. This is the first epidemiologic study in Northeastern British Columbia on the association between proximity/density of wells and birth outcomes. Our results provide some evidence of a potential association with more preterm births and reduced birthweight, but effect estimates did not match expected dose-response relationships.

1651 Pathogenicity and Antimicrobial Susceptibility of *Aeromonas hydrophila* Isolates from Fish in Abuja, Nigeria

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The adverse effects of *Aeromonas* species have been reported in aquatic organisms, humans and food spoilage globally especially in developing countries like Nigeria with *Aeromonas hydrophila* as the most common and toxin producing species. Pathogenicity studies of infections caused by aeromonads is often neglected and under reported which necessitated this study to establish the virulence factors and effectiveness of selected antimicrobials on isolates obtained from fresh and ready to eat fish in Abuja-Nigeria. Fish samples were collected and processed from major markets in some Area Councils in Abuja for conventional culture and identification methods at the Public Health Bacterial Zoonosis Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Kaduna State. The presumptive *Aeromonas* hydrophila isolates were further subjected to extracellular virulence factors determination assay based on protease production, DNase production, hemolysin production and enterotoxin production using Suckling Mouse Test (SMT). Antimicrobial susceptibility testing on isolates was also conducted using Kirby Bauer disc diffusion method. Results showed that all the isolates produced 100% DNase, gelatinase, and hemolysin while few (40%) produced exotoxins. Distended abdominal-body weight ratio and black necrotic gastrointestinal tract lesions further confirmed evidence of enterotoxins. Isolates also dissipated array of resistance to different antimicrobial agents. These virulent factors could have been involved in the pathogenicity and resistance of isolates. In conclusion, *Aeromonas* hydrophila is highly pathogenic and resistant to commonly used antibiotics. Hence, the need create public awareness on the health implication of this organism and design of appropriate measures for *Aeromonas* menace control in Abuja and Nigeria.

1652 Automated Mining of Vaping-Associated Health Effects Reported in Online Forums

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Vaping-associated pulmonary illness (VAPI) has reached epidemic proportions in the United States. To better understand VAPI, we used computer automated methods to extract and analyze over 41,000 posts from a major EC online forum. The posts spanned 7.5 years and were used to identify the health effects associated with EC use. Extracted data were annotated with a set of medical concepts from the Unified Medical Language System (UMLS), using a modified version of the MetaMap tool. Posts were used to analyze symptoms (undiagnosed conditions) and disorders (physician diagnosed terminology) associated with EC use. Symptoms and disorders were then categorized into 12 organ systems/anatomical regions. Data were further analyzed for: (1) frequency of symptoms and disorders, (2) sentiment, (3) changes in reported health effects between 2008 and 2015, (4) linkage between symptoms, and (5) similarities between our data and VAPI case report symptoms. Symptoms and disorders were most often reported in the neurological, respiratory, digestive, integumentary, and mouth/throat systems, over the 7.5-year period studied. The most common symptoms were: headaches (N=939), coughing (N=852), pain in throat (N=643), dermatitis (N=565), and heartburn (N=327). The most commonly reported disorders were asthma (N=916), pharyngitis (N=565), chronic obstructive pulmonary disorder (N=471), dehydration (N=403), aptyalism (N=377), and pneumonia (N=367). Overall, most symptom and disorder posts contained negative sentiment across all years. Several symptoms were linked (e.g., coughing and headache). Additionally, many commonly reported symptoms in this dataset are present in the current VAPI cases. Online forums are a unique repository of data that can track health effects over time. This is the first study to use automated methods to retrieve EC health data online, and it demonstrates the importance of medical Internet surveillance. Our data agree well with the symptoms of reported for VAPI cases. This suggests that health problems have existed among vapers for at least 7.5 years, although this has not been widely recognized by the medical community until now. Our data also suggest that there are many other EC users with pulmonary illness due to vaping who are not yet part of the statistical data reported by the Centers for Disease Control.

1653 Exposure Assessments and Cancer Epidemiology: Will the Past Work for the Future?

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Of the many chemicals assessed under US EPA's Integrated Risk Information System (IRIS) program, a small number have been classified as known human carcinogens. The classifications are based in part on epidemiological studies with various types of exposure assessments. We reviewed the underlying epidemiology studies that support ten carcinogen determinations and examined methods used in the exposure assessments. We found that the studies have four common features: (1) predominantly retrospective, (2) occupational-based, (3) presumably high exposures (compared to general population exposures) and (4) exposure assessments including limited to no chemical measurement data or methods documentation. Chemical-specific examples (e.g., nickel subsulfide, benzene, arsenic) highlighting various issues (e.g., peak versus average exposures, temporal variability) with exposure assessments in research used as part of IRIS assessments will be described. Although many recent meta-analyses and literature reviews on carcinogens have not uniformly incorporated quality appraisals, some agencies are presently using formal, systematic approaches to assessing study quality as part of decision-making and this is becoming the state-of-the-art for both regulatory decision-making as well as literature reviews. Current methods and approaches developed for conducting these kinds of appraisals will be discussed. These include systematic review approaches developed as part of EPA's IRIS program and Toxic Substances Control Act risk evaluations as well as approaches developed by the National Institute of Environmental Health Sciences. These examples can assist investigators who wish to develop studies that consider newly emerging risk of bias assessments.

1654 E-waste in Ghana: An Assessment of Health and Psychosocial Effects on Communities in and around Agbogbloshie

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Electronic waste (e-waste) is a term coined to describe the end-of-life remnants of electronic equipment. This form of waste, shipped mostly from high-income to low-and middle-income countries (LMICs), is used particularly for mining of valuable metals - and thus is an integral part of the economy of many LMICs. This type of waste, however, has also become an emerging health concern. E-waste processing poses a threat to human health, due to lack of personal protective equipment, unsanitary conditions, and improper handling of hazardous metals. One area in Ghana in particular -Agbogbloshie- is home to one of the largest e-waste sites in the world. Past studies in this area have discovered elevated levels of organochlorines, pesticides, and heavy metals in the environment. To better understand the health and psychosocial effects of e-waste, a study involving 623 individuals in the e-waste enterprise and those who live close to the e-waste site in Agbogbloshie was conducted in October 2018. Findings from the surveys revealed that 51% of respondents experienced more than one symptom associated with heart disease, and 67% experienced symptoms associated with lung disease. The study also found that 27% of those working in the e-waste business experienced regular periods of work-related stress, 83% of the workers from this study said they knew the dangers of e-waste, and over 90% of workers would be interested in getting more information on how e-waste relates to their health. Given that cardiovascular and lung-related diseases are among the leading causes of death globally, there is increasing concern about how e-waste may contribute to the overall health burden of Ghana and other countries that are also economically-supported by e-waste. Given the increase of e-waste processing sites around the world and the toxicity of most e-waste constituents such as cadmium, nickel, arsenic and plastics which are often burned after the pile is 'scrounged', highlighting the health implications associated with exposure to e-waste, possible solutions and policy changes will help better understand and educate the populations being impacted. *Supported by NYU Global College of Public Health Affinity Grant; NYU NIEHS P30ES000260-55.*

1655 Antibacterial Effects of Antiretrovirals: Implications for Aquatic Systems and Public Health

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Antiretroviral (ARV) drugs are used as treatment or prophylaxis for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) by over 23 million persons worldwide. Nucleoside reverse transcriptase inhibitors (NRTIs), one class of ARVs, are analogs of the biological nucleosides adenosine, guanosine, thymidine, and cytidine and are designed to inhibit the HIV reverse transcriptase enzyme. One NRTI in particular, azidothymidine (AZT), a thymidine analog, possesses not only antiretroviral capacity but also antibacterial capacity. However, little is known about the antibacterial capacity of other NRTIs. In this study, we compared the impacts of AZT and six other common NRTIs on the growth of the model bacterium *Escherichia coli* in culture. Effects of NRTIs individually and in combination were assessed at concentrations ranging from 0.001 ug/ml to 100 ug/ml over 48 hours. AZT concentrations as low as 0.001 ug/ml decreased the growth of *E. coli* by one-third within 5 hours of incubation compared to controls. The NRTIs lamivudine, stavudine, and didanosine also demonstrated antibacterial capacity. *E. coli* treated with these NRTIs showed reduced growth compared to controls within 10 hours at concentrations between 0.1 and 100 ug/ml. Interestingly, *E. coli* quickly developed resistance to stavudine, didanosine, and lamivudine, and resistance to one NRTI conferred resistance to other NRTIs when challenged despite chemical structural diversity, suggesting the mode of action of NRTIs on *E. coli* may be similar. Previous work has revealed presence of ARVs in wastewater, river water, and drinking water throughout the world. ARVs have the potential to affect the natural bacterial and viral ecosystems of aquatic environments as well as the beneficial biota of the human gut microbiome. As this work highlights, the antibacterial effects of ARVs, and the potential for developing resistance, have important implications for the public health, from water systems to human physiology.

1656 Respiratory and Cognitive Outcomes of Puerto Rican Children Exposed to Traffic-Related Air Pollutants

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Traffic-related air pollution (TRAP) emissions are a major source of air pollutants in urban areas. Puerto Rican children are considered a high-risk population due to high poverty rates, high asthma prevalence, and low academic achievement. Thus, the main goal of this study is to evaluate the impact of TRAP in lung function and neurocognition in Puerto Rican children living in urban areas. Specifically, to examine short- and long-term impact of living near two sources of TRAP in lung function and neurocognitive processes of 6 to 9 years-old schoolchildren. We conducted an exploratory longitudinal study (3 years) in which we recruited 46 children living upwind and downwind TRAP sources. During the academic year, lung function was measured on a weekly basis using a spirometer; and neuropsychological assessments were conducted every semester with the Spanish translation of the Cognitive Assessment System 2nd edition (CAS2). Environmental monitoring included meteorological variables (temperature, relative humidity, wind speed and direction) and pollutant concentrations ($PM_{2.5}$, CO_2, VOCs). Parents and legal guardians filled a questionnaire about health, environmental factors and sociodemographic information. The median age at recruitment was 6.3 years. Participants were 57% female, 100% Hispanic, and mostly of low socioeconomic status (79.5% from households with incomes below $25,000 and 68.9% enrolled in public health insurance). Lung function measurements revealed lung volumes lower than predicted for their age, sex, height, and ethnicity based on NHANES III reference values. Lung volumes were consistently lower in participants living downwind TRAP sources, mostly for forced expiratory volume in 1 second (FEV1). Similarly, children scored below average in all cognitive tests, mainly in the planning and successive processing assessments. Overall results suggest impairments in respiratory and cognitive health of Puerto Rican children living near TRAP sources.

1657 Indoor Dust Assessment of Endotoxin and B-Glucans Levels in the Aftermath of a Major Hurricane

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Hurricane-related water damage can lead to an increase in indoor growth of microorganisms such as bacteria and mold. Indoor exposure to these microbes and its components, such as endotoxin and β-glucans, can cause respiratory illness such as allergic rhinitis, exacerbation of asthma, hypersensitivity pneumonitis, and respiratory infections. Therefore, the purpose of this study is to characterize the potential risk of exposure to endotoxin and β-glucans in occupational settings that sustained water-damage due to the landfall of Hurricane María in Puerto Rico. We collected indoor dust from horizontal (i.e. desks) and vertical surfaces (i.e. walls) 21 months post-hurricane since Puerto Rico is still not fully recovered. Dust samples were obtained from buildings located in municipalities with varying degrees of water-damage. A total surface area of 100cm^2 was assessed for desks and 900cm^2 for walls. Each area was sampled in triplicate. Samples were collected with sterile foam swabs, extracted by sonication with endotoxin-free water, and analyzed for endotoxin and β-glucans using the kinetic Limulus Amebocyte Lysate and Glucatell assays, respectively. Results revealed that β-glucans were >5,000 pg/m^2 in all samples. Endotoxins concentrations varied between 0.001 EU/m^2 and 1000 EU/m^2. Higher concentrations of endotoxin (up to 700X) were observed in areas that presented more damage from the hurricane when compared to the other sampling sites. Therefore, our results suggest that almost two years after the hurricane, workers are at risk of being exposed to endotoxin and β-glucans concentrations that can induce adverse health effects.

1658 Maternal Serum Folate Concentrations Modify the Association between Prenatal Arsenic Exposure and Birth Outcomes in the Biomarkers of Exposure to ARsenic (BEAR) Cohort

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Women of reproductive age, especially those planning to become pregnant, are advised to consume folate for the prevention of neural tube defects among offspring. As a methyl-donor nutrient, folic acid may also be critical to the metabolism of inorganic arsenic (iAs), a ubiquitous environmental contaminant and carcinogen commonly found in drinking water. iAs is biotransformed to monomethylated (MMA) and dimethylated (DMA) arsenic through the addition of methyl groups, and folate supplementation among adults has been shown to improve metabolism efficiency. We previously identified associations between iAs exposure during pregnancy and birth outcomes in the Biomarkers of Exposure to Arsenic (BEAR) cohort (n = 200). To identify whether these associations are attenuated by increased folate consumption, multivariable-adjusted linear regression models were fit. Likelihood ratio tests were implemented to evaluate the goodness-of-fit of interaction models as compared to main effects models. Additionally, to compare the magnitude of each association according to folate status, strata-specific mean differences and 95% confidence intervals were estimated. Overall, the prevalence of folate deficiency, defined as less than 9 nM, was low (< 2%). Still, significant (p < 0.10) effect modification by folate was observed on the additive scale for the associations between U-tAs (β: 2.89, p: 0.06), U-MMAs (β: 41.70, p: 0.07), and U-DMAs (β: 3.19, p: 0.06) and birth weight (g) and between DW-iAs (β: 0.01, p: 0.07), U-iAs (β: 0.19, p: 0.03), and U-MMAs (β: 0.19, p: 0.02) and head circumference (cm). Despite a high prevalence of folate sufficiency, increased folate uptake modified the association between drinking or urinary arsenical metabolites and birth outcomes. The results from this cross-sectional study suggest that infants born to women living in regions with high iAs-contaminated drinking water may benefit from increased prenatal folic acid supplementation, at levels exceeding current recommendations.

1659 ToxPi*GIS: Translation of Multi-Stream Data into Interactive Visual Profiles within a Geospatial Context

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Creating effective policies to support public health and respond to environmental emergency-related events require accessible tools and models that address complex data on exposures and relevant pre-existing stressors. To support this objective, we have created a ToxPi*GIS tool that integrates diverse data streams into interactive profiles atop geographic information system (GIS) layers. The Toxicological Prioritization Index (ToxPi) framework creates visual profiles that communicate scores and an accompanying statistical framework for comparison, clustering, and evaluation of model sensitivity to component data streams. The ToxPi*GIS tool combines this profiling framework with ArcGIS to create a web interface that can handle data from any geographic region of interest. Here, we present results from analysis of environmental data on contaminants, health, socioeconomics, and flooding for census tracts in the greater Houston, TX area. The ToxPi*GIS results highlighted areas of high concern (statistically significant collections of census tracts according to the Getis-Ord Gi* statistic) in our integrated model. By comparing ToxPi profiles across all tracts to identify clusters of multivariate profile similarity, we also found geographically disparate areas that showed similar drivers of concern. Thus, ToxPi*GIS helps identify both geographically-adjacent and geographically-distinct clusters, where integrated profiles can be assessed to promote creation of targeted, effective community policies. Users can upload models for any geographic area of interest, explore global patterns, perform multi-criteria filtering, and export results for sharing from the free, interactive ToxPi*GIS interface at https://toxpi.org/gis.

1660 Association between Thyroid Cancer Risk and Exposure to Heavy Metals: A Systemic Review and Meta-Analysis of Cohort Studies

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Thyroid cancer is one the most common endocrine malignancies whose incidence rate has rapidly increased in the past few decades. However, the risk factors contributing to the rise of thyroid cancer remain not well-elucidated. Animal studies and clinical evidence have suggested that increased exposure to heavy metals could disrupt thyroid homeostasis and function, alter peripheral metabolism, and accelerate thyroidal tumorigenesis. Nevertheless, epidemiological evidence regarding effects of heavy metals on thyroid carcinogenesis has been inconsistent. Therefore, the aim of this study was to perform a meta-analysis to assess the potential effects of heavy metal exposures on thyroid cancer risk. A comprehensive literature search was conducted to identify eligible studies based on the guidelines of MOOSE and PRISMA. Summary relative risk (RR) and confidence intervals (CI) were used to compute thyroid cancer risk by random-effects models. The heterogeneity was assessed for statistical significance using I^2-based-Cochrane Q test. Overall, 4917 bibliographic records were identified; 8 cohort studies were eventually included for analysis. Exposure to heavy metals is associated with a significant rise in thyroid cancer risk (RR = 1.40, [CI 1.16- 1.69], $p < 0.001$). Subgroup analysis revealed an increased thyroid cancer risk from heavy metal exposure in both sexes, with the effect more pronounced in females than in males (RR = 2.01 [CI 1.73 - 2.34] vs RR = 1.86 [CI 1.63 - 2.12], respectively). Elevated thyroid cancer risk is associated with exposure to a mixture of multiple heavy metals simultaneously (RR = 1.83, [CI 1.62- 2.05], $p = 0.003$), while more evidence is needed to conclude such association with exposures to single heavy metals (RR = 1.88, [CI 0.95- 3.71], $p = 0.2$). Pooled relative risks for environmental exposures and occupational exposures were 1.88 ([CI 1.62 - 2.16], $p < 0.001$) and 1.69 ([CI 1.40 - 2.03], $p = 0.532$), respectively. No publication bias was detected. Our study indicates that heavy metal exposure, both environmental and occupational, is associated with an elevated thyroid cancer risk. Therefore, heavy metal exposures should be further evaluated as a potential risk factor for the global upward trend of thyroid cancer incidence.

1661 The Associations between Primary Ovarian Insufficiency and Environmental Pollutants in Korea

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Sponsor: J. Park

The prevalence of primary or premature ovarian insufficiency (POI) is about 1% in women before the age of 40. As the prevalence of POI continues to increase, exposure to environmental pollutants is being discussed as one of the factors causing POI. The purpose of this study is to investigate the associations between environmental pollutants and POI in Korea. The subjects were one hundred and fifty six women who were recruited in Anam Korea University Hospital (IRB No.2016AN0251). Because POI is characterized by increase of follicle stimulating hormone (FSH) with decrease of anti mullerian hormone (AMH), the subjects were evaluated by the serum level of FSH and AMH with other gynecological examinations. The subjects were classified into three groups as POI patients, borderline POI patients, and non-POI normal subjects. Their urinary concentrations of metabolites of phthalates, bisphenol A, volatile organic compounds (VOCs), and polycyclic aromatic hydrocarbon (PAH) were determined for the evaluation of exposure to environmental pollutants. POI patients showed highest concentrations of phenyl glyoxylicaid (PGA) and 1-hydroxypyrene (1-OHP) among three POI/normal groups. And subjects were stratified into 26-30, 31-35 and 36-40 years old age groups to compare urinary concentrations of pollutants among three POI/normal subjects in the same age group. All age subgroups showed that POI patients had the highest 1-OHP levels among three POI/normal subjects. And both mandelic acid (MA) and PGA concentrations of POI patients were significantly increased only in the 31-35 years old age group. To evaluate the association of AMH levels and exposure to environmental pollutants only in normal subjects, the subjects were divided into high and low exposure groups based on each urinary metabolites of pollutants. Significantly lower levels of AMH showed in high exposure groups of 2,4-Dichlorophenoxyacetic acid (2,4-DCP), N-Acetyl-S-(benzyl)-L-cysteine (BMA) than low exposure groups. And decreasing trend of AMH levels were found in high exposure groups of phthalate, BPA and VOC than low exposure groups. Our study suggests that several environmental pollutants might be risk factors to POI or decrease of AMH.

1662 Identification of Metal Mixtures in Pregnant Women and Associations with Offspring Weight and Blood Pressure Outcomes at 4-6 Years of Age

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Toxic metals including arsenic, cadmium, and lead are chemical pollutants that often co-occur in the environment. Prenatal exposure to heavy metals can affect proper fetal growth and development, which is a risk factor for obesity and its co-morbid conditions. Our study objectives are to: 1) identify metal combinations present in the blood of pregnant women in our birth cohort; and 2) determine whether prenatal exposure to metals either individually, or as a mixture, are associated with an increased risk for offspring obesity and elevated blood pressure. To address these questions, we measured the blood concentrations of 24 metals which include heavy, trace, and potentially nutritive metals in n=310 women during their first trimester of pregnancy and applied both unsupervised and supervised statistical methods. We used Factor Analysis to identify metal mixtures in maternal blood and ran sex-stratified regression models including Partial Least Squares and Elastic Net to assess associations between potential key metals and their effects on weight and systolic/diastolic blood pressure in offspring between 4-6 years of age. We identified consistent and significant ($p<0.05$) associations between metals, including arsenic and chromium, and increased weight and blood pressure. Further analysis and characterization are needed for metals speciation and to ascertain exposure sources.

1663 Applying the Key Characteristics Approach for Identification and Analysis of Toxicological and Mechanistic Evidence on Benzo[a]Pyrene-Induced Male Reproductive Toxicity

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Hazard evaluation of chemical-induced adverse responses in the male reproductive system involves qualitative analyses of epidemiological, toxicological, and mechanistic studies. Inclusion of mechanistic evidence in this analysis can inform the biological plausibility and human relevance of effects observed in experimental models. However, the diversity of research methods and models, and the variety of proposed and known pathways for chemical-induced reproductive toxicity can add difficulty to the practice of screening and analyzing mechanistic outcomes. The ten key characteristics of carcinogens provide a tool for identifying, organizing, and evaluating chemical-specific mechanistic evidence. Here we apply the eight key characteristics of male reproductive toxicants to identify and analyze the toxicological and mechanistic evidence on benzo[a]pyrene (B[a]P)-induced male reproductive effects. A literature search was performed to capture mechanistic studies and literature inventories were developed to extract evidence from the identified studies and facilitate the qualitative review process. Preliminary analysis suggests that several key characteristics are involved in B[a]P-induced male reproductive effects, including: alters somatic cell development, functions, or death; alters germ cell development, function, or death; is genotoxic; induces oxidative stress alters production and levels of reproductive hormones; and alters hormone receptor levels/functions. A qualitative evaluation is underway to identify potential pathways of B[a]P-induced male reproductive toxicity and ascertain the biological plausibility and human relevance of the effects reported in experimental analysis. The B[a]P case study demonstrates that the key characteristics approach serves as a practical and objective tool for identifying, organizing, and evaluating chemical-specific mechanistic studies and evidence on male reproductive toxicity. *Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.*

1664 Evaluation of Hair Breakage following Repeated Grooming of a Personal and Care Cosmetic Product

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There have been reported adverse event claims associated with the use of WEN by Chaz Dean (WCD) cleansing conditioners, resulting in an investigation by the US Food and Drug Administration (US FDA). Specifically, users of WCD cleansing conditioners have reported hair loss, hair breakage, and skin irritation reactions. Therefore, this analysis performed a dry repeated grooming study to assess if application of WCD cleansing conditioners was associated with changes in hair tensile strength and hair breakage. Ten European medium brown hair tresses (9% bleached, 3.0 g, 8" length, 1" wide) were used in each treatment group. The control hair was treated with 15% sodium lauryl ether sulfate, typical control conditions, for a 30 seconds lather and 30 seconds rinse, while the test hair was treated with the top 3 selling WCD cleansing conditioners (Sweet Almond Mint, Lavender, and Pomegranate) for 10 minutes (to simulate a typical shower application), followed by a 30 seconds rinse. Hair was equilibrated overnight and then was groomed using an automatic comber for 2,000 cycles, with broken hair fibers collected every 200 cycles. Hair treated with WCD cleansing conditioners had a statistically significant fewer number of broken hair fibers in comparison to the control. The mean number of broken fibers after 2,000 brush strokes were: 43 fibers (control), 22 fibers (Sweet Almond Mint), 28 fibers (Lavender), and 28 fibers (Pomegranate). The WCD cleansing conditioners exhibited beneficial qualities of acting as surface lubricants that reduced stress from grooming, and thus, reducing hair breakage. Overall, findings from this analysis provide evidence that under the conditions tested, the use of WCD cleansing conditioners is not associated with hair breakage following repeated grooming.

1665 Mucosal Damage and Gamma-H2AX Formation in the Rat Urinary Bladder Induced by Aromatic Amines with Structures Similar to That of *o*-toluidine

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Although some of aromatic amines are closely associated with the risk of bladder cancer, the bladder toxicity and carcinogenicity of many aromatic amines remain unknown. Our recent study demonstrated distinct differences in the mechanisms of mucosal damage in the urinary bladder of rats receiving *o*-toluidine and *o*-anisidine, which are bladder-carcinogenic aromatic amines. In the current study, we examined histopathological changes and formation of γ-H2AX, a biomarker of DNA damage, in the urinary bladder of rats in order to examine the bladder toxicity and carcinogenic potential of several aromatic amines with structures similar to that of *o*-toluidine. Methods: Six-week-old male F344 rats were administered 1.0% 4-chloro-*o*-toluidine (4-CT), 0.5% 5-chloro-*o*-toluidine (5-CT), 0.5% *o*-aminoazotoluene (AAT), 1.0% 2-aminobezyl alcohol (ABA), or 1.0% *o*-acetotoluidine (*o*-AT) in the diet for 4 weeks. Five animals in each group were necropsied on day 2 and weeks 1, 2, and 4, and histopathological and immunohistochemical analyses were performed. In the 4-CT and *o*-AT groups, bladder lesions similar to those induced by *o*-toluidine, including hemorrhage, interstitial necrosis, perivascular inflammation, granulation tissue formation, and simple hyperplasia, were observed from week 1. In the AAT and ABA groups, simple hyperplasia without degenerative and inflammatory changes was detected from week 1, similar to *o*-anisidine. γ-H2AX-positive epithelial cells in the urothelium were significantly increased in the 4-CT, AAT, ABA, and *o*-AT groups from week 1 or week 2. No apparent bladder lesions or significant increases in γ-H2AX formation were observed in rats receiving 5-CT, despite having a structure similar to that of 4-CT. Our results suggested that 4-CT, AAT, ABA, and *o*-AT may induce DNA damage in the urinary bladder of rats. Moreover, histopathological examination revealed that there were two distinct mechanisms of bladder mucosal damage, similar to *o*-toluidine (4-CT and *o*-AT) and *o*-anisidine (AAT and ABA), respectively. Further studies are needed to clarify the molecular mechanisms of bladder toxicity associated with these aromatic amines.

1666 Stepwise Safety Evaluation Process for Natural Complex Substances

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The Research Institute for Fragrance Materials, Inc. (RIFM) has evaluated the safety data for fragrance materials for over 53 years. The process to evaluate fragrance materials and available data has evolved as new scientific approaches emerge. Since 2013, RIFM has focused its efforts to evaluate discrete, synthetic materials, but in 2019 and beyond the primary focus has shifted to evaluating Natural Complex Substances (NCS). A common misconception is that "natural" inherently means safe and simple, but determining the safety of NCS is an issue facing many industries. RIFM will leverage the latest science as well as the extensive knowledge compiled over the years concerning the components of NCS. The basic premise is the same as for discrete fragrance materials in that all of the same endpoints (Genotoxicity, Phototoxicity and Photoallergenicity, Skin Sensitization, Repeat Dose Toxicity, Developmental and Reproductive Toxicity, Local Respiratory Toxicity, as well as Environmental Risk and Hazard) will be addressed (Api, et al., 2015). A series of decision trees, reflecting advances in approaches in risk assessment of mixtures as well as classical toxicological methodologies will follow a similar four-step process with testing only as a last resort: Step 1) evaluate the available data on the NCS, 2) can Threshold of Toxicological Concern (TTC) be applied, 3) can the NCS risk assessment be achieved on a component basis, 4) data may need to be generated. For each endpoint, a tiered approach was developed based on this 4-step premise. Using *in silico* tools, RIFM examined NCS similarities based on plant part, processing (distillation, mechanical extraction, and solvent extraction), and composition of materials across 81 plant families in an effort to address data gaps. Data generated from the Creme RIFM Aggregate Exposure Model for over 900 fragrance NCS demonstrates that dermal exposure is the primary route of human health exposure, and over a third of the materials are below the most conservative TTC limits. This process provides a comprehensive and robust safety assessment of NCS. Api, et al., 2015. Criteria for the Research Institute for fragrance materials, Inc. (RIFM) safety evaluation process for fragrance ingredients. Food Chem. Toxicol. *82, S1-S19.*

1667 A Safety and Effectiveness Evaluation of a Callus Softener Containing Potassium Hydroxide

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Limited safety information has been described in the peer-reviewed literature for callus softening products containing potassium hydroxide. This pilot human use study evaluated the safety and effectiveness of a commercially available callus softener, containing less than 10% potassium hydroxide by weight. Baseline callused skin was scored (grade 1-4) on each study participant's feet (n=10). Participant's feet were soaked and then a licensed manicurist applied a callus softener product to the right foot, which remained on callused skin for 3 to 5 minutes (no callus softener was applied to the participant's left foot). Both feet were then wiped with a wet towel, and a foot rasp was used to file the callused skin, beginning on the left foot. Callused skin was scored and participant's feet were evaluated by a physician immediately post use, 1-day post use, and 1-week post use for the presence/absence of skin irritation, adverse skin reactions, and chemical burns. No adverse events were reported by study participants or the physician for all evaluation time points. Each participant's highest callus grade score on the treated foot either improved or remained the same following product use (compared to baseline). Mean callus grade scores were 1.75 at baseline, 1.55 immediately post use, 1.25 1-day post use, and 1.50 1-week post use. Results from this pilot study suggest that callus softening products containing less than 10% potassium hydroxide are likely to be safe and effective products under intended use scenarios of 3-5 minute application times, as dictated by product label instructions.

1668 Glyphosate Safety Assessment through Ames Test

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Glyphosate is widely-used herbicide to control unwanted grass and broadleaf weeds. It is one of the active ingredients in Roundup, a ubiquitous organophosphorus compound. A recent lawsuit in California associating glyphosate with lymphoma generated media interest regarding the safety of glyphosate. US EPA and IARC (International Agency for Research on Cancer) have conflicting views associated with the risks related to glyphosate. We conducted the Ames test, a gold standard test the FDA uses as a part of a battery to access mutagenicity of test compound. Four different *Salmonella* Typhimurium strains were used to screen the genotoxicity of glyphosate. Treatment with and without S9 activation were conducted to understand the toxicity potential under metabolic activation. OECD (Organization for Economic Co-operation and Development) protocol 471 was followed to perform this assay. Two strains of *Salmonella* Typhimurium, 1535 and 1537 showed higher number of revertants with S9 activation. At higher concentrations, 500 and 5000 µg/ml, colony count were decreased as compared to controls, indicating toxic effect of glyphosate. The remaining two strains, 98 and 100, did not show toxic potential of the glyphosate. Overall, Ames test using these four strains did not reveal mutagenic potential of glyphosate. However more studies including additional *Salmonella* enterica and *E. coli* strains are required to explain risk associated with glyphosate. *In vitro* and *in vivo* studies using various cell lines and animal models respectively can shed more light on risk associated with glyphosate.

1669 Human Health Hazard Assessments of Alkyl Dimethyl Benzyl Ammonium Chloride and Didecyl Dimethyl Ammonium Chloride

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Quaternary ammonium compounds (Quats) are a large group of permanently charged cationic chemicals that are used in a variety of consumer and industrial products. Alkyl (C12, C14, C16) dimethyl benzyl ammonium chloride (ADBAC) and didecyl dimethyl ammonium chloride (DDAC) are two of the most widely used biocidal Quats that are representative of two structurally distinct classes. In Europe, biocidal Quats are regulated by ECHA under the Biocidal Products Regulation (BPR), while US EPA regulates biocidal Quats under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). Both agencies require substantial amounts of human health hazard data to support registration. Available data indicate that ADBAC and DDAC are poorly absorbed via the oral and dermal routes (≤10%), are not systemically distributed, undergo limited metabolism, and are primarily excreted in the feces. ADBAC and DDAC are not dermal sensitizers, genotoxic, carcinogenic, or specific developmental or reproductive toxicants. ADBAC is acutely toxic via the oral (LD_{50}s = 154 to 550 mg/kg), dermal (LD_{50}s = 704 to 3,413 mg/kg), and inhalation (0.054 mg/L < LC_{50} < 0.51 mg/L) exposure routes. DDAC is acutely toxic via the oral (LD_{50}s range from 238 to 329 mg/kg) and inhalation (LC_{50} = 0.07 mg/L) exposure routes, but not via the dermal route. In terms of inhalation toxicity and potential exposure via this pathway, ADBAC and DDAC are both considered nonvolatile, and do not readily become airborne during most applications. Both ADBAC and DDAC are irritating/corrosive to the skin, and can cause non-specific toxicity in subchronic and chronic repeated dose oral toxicity studies. Reported NOAELs for ADBAC and DDAC range from 3.7 to 188 mg/kg-day and 10 to 93.1 mg/kg-day, respectively, in subchronic and chronic toxicity studies conducted with mice, rats, and beagles. Adverse effects associated with reported NOAELs are consistently characterized by local irritation of the stomach, reduced body weight gain, and reduced food consumption, indicative of portal-of-entry effects commonly associated with irritating/corrosive chemicals. Based on available data, local irritation is the main hazard associated with ADBAC and DDAC, which is consistent with hazard assessments conducted by ECHA and US EPA under BPR and FIFRA, respectively.

1670 Effect of Exposure to Commercial Hair Care Products on Primary Human Hair Follicle Dermal Papilla Cells

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Dermal papilla cells (DPCs), which are often described as stem cell-like cells, are specialized mesenchymal cells located inside human scalp hair follicles. DPCs play a key role in regulating development and growth of both the hair and hair follicle and have remained a topic of interest in the last few decades. More importantly, any adverse effect on DPCs may directly impact the growth and health of the entire hair. Recent allegations of hair loss due to use of hair care products have prompted interest in the potential impact on DPCs. Although no validated assays using human hair follicle dermal papilla cells (HFDPCs) exist, we designed an exploratory assay to evaluate the potential effects of product exposure on HFDPCs. Primary HFDPCs isolated from a female donor were cultured and treated with a range of dilutions of three commercial hair care products. These dilutions represent concentrations above that which is expected to come into contact with the hair follicles on the human scalp during product use (e.g., in the shower). In order to simulate a typical product use scenario, HFDPCs were treated for 15 minutes each day for five consecutive days. Repeated exposure to ≤ 0.001% hair care product did not significantly induce cytotoxicity compared to untreated (media only) controls, while significant cytotoxicity was observed upon treatment with 0.02% sodium dodecyl sulfate (SDS) (positive control). Additionally, no significant differences in cell morphology were observed following repeated exposure to the hair care products. Overall, this study indicates that exposure to commercial hair care products at the tested concentrations does not adversely impact overall viability and morphology of HFDPCs.

1671 Feasibility Assessment of Subcutaneous Radio-Telemetry Device Implantation in Cynomolgus Monkeys

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With the goal of potentially reducing the trauma caused by surgical implantation of telemetry units without negatively impacting the quality of information obtained from studies involving such implants, Altasciences investigated the feasibility and quality of data capture of two different methods of telemetry device implantation in non-human primates (NHPs): the standard intra-abdominal implant method vs. a less invasive subcutaneous implant method. Four animals (2 per gender) were assigned to each group. Animals were implanted with radio-telemetry transmitters to allow for continuous capture of cardiovascular and body temperature data. All animals recovered for a minimum of 21 days prior to the beginning of monitoring. Rectal temperature and clinical pathology (hematology, coagulation, and serum chemistry) were also captured on a weekly basis. Telemetry data obtained in weeks 1 through 6 showed no marked differences in cardiovascular readings between intra-abdominal and subcutaneous implants. Blood pressure, heart rate, and ECG were all found to be within normal ranges for both groups. There was a notable difference in body temperature readings obtained between the two implant sites during the first 3 weeks of monitoring. Mean temperatures recorded in the intra-abdominal implants were 1.5 degrees lower than rectal temperatures recorded on the same day, while temperatures in the subcutaneous implants were often 3 to 4 degrees lower. There appeared to be a gradual loss of accuracy over time in intra-abdominal tem-

perature readings after week 3. Minimal changes in clinical pathology observed during the post-implantation phase in both groups were consistent with low-grade inflammation. The pattern and extent of the alterations were comparable across implantation sites, indicating little difference in surgical trauma between methods. The feasibility of sustaining subcutaneous implant placement was limited by animal size. Larger male animals (4.5-5 kg) tolerated the subcutaneous implants well while the smaller females (~3 kg) did not due to the lack of a subcutaneous space that would allow the device to sit comfortably. One female was euthanized after 5 weeks of monitoring due to repeated exposure of the device. Subcutaneous telemetry implants appear to be a viable alternative to intra-abdominal implants in NHPs of sufficient size, but are not ideal for smaller animals.

1672 Does the Cytotoxicity of E-liquids Increase with Age?

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Many users of e-vapor products favor highly flavored e-liquids. Such e-liquids are often contain aromatic aldehydes and other species that react with the glycerol and propylene glycol, the major constituents of e-liquids, to form acetals and other reaction products. E-liquid composition at the time of use depends, in part, on time since manufacture and what the user does with the bottles of e-liquid after purchase. Is it loaded into the vaping device as is, or is it modified by the user by steeping it by placing the container of e-liquid in an ultrasonic bath before use (a process that can cause chemical changes)? Moreover, there is a trend to providing e-liquids in larger containers than were formerly available. 120-mL bottles are common and one manufacturer is selling e-liquids in 1-liter containers. At 5-mL per day, are they any changes over the 24 days the 120 mL of e-liquid are used. Research to be presented covers these questions as well as addition of acids (so-called nic salts) for ten commercial e-liquids. For example, two vanilla-flavored e-liquids (one with nicotine salts and the other without nicotine) were forced aged (11 days at 64^{0}C), and the chromatographic (HPLC with several columns and conditions) and cytotoxic (NRU with CHO cells done by commercial laboratory) properties compared with each other and their unaged controls. Differences in the chromatographic processes were not reflected in the NRU data. A new sample of a highly flavored e-liquid in a 120-mL bottle was received from the manufacturer, and 5-mL aliquots were removed daily and stored in the freezer until the bottle was empty. Changes in composition were typical for those observed in steeping experiments; however, changes in cytotoxicity were unremarkable. Based on the data obtained changes in chemistry during aging are not reflected in the cytotoxicity assay.

1673 Therapeutic and Safety Evaluation of Flex Choice in Moderately Osteoarthritic Dogs

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Osteoarthritis (OA) is a chronic inflammatory degenerative joint disease that inflicts humans and animals alike. Currently, >20% of the adult and 80% of the geriatric dog population in the US (>90 million) suffer from OA. The pathophysiology of OA is very complex as it involves multiple mechanisms and molecular pathways. Currently, there are many choices to manage OA, but many veterinarians choose nutraceuticals because of lesser or no side effects compared to pharmaceuticals. The present investigation was undertaken to assess anti-arthritic efficacy and safety of FLEX CHOICE™ chews (a product of Clinics Choice, LLC) in dogs with moderate OA. FLEX CHOICE™ is composed of krill oil, hyaluronic acid, astaxanthin, *Boswellia serrata* extract, green lipped mussel, and iron transport tocopheryl polyethylene glycol succinate (ITPGS). Dogs with OA received FLEX CHOICE™ chews b.i.d. for 150 days. Each month, dogs were given a full exam and were evaluated for arthritic pain (overall pain, pain upon limb manipulation, and pain after physical exertion) using the Glasgow scoring system, CBC, and serum biomarkers of liver (bilirubin, ALT, and AST), kidney (BUN and creatinine), and heart and skeletal muscle (CK) functions. Dogs receiving FLEX CHOICE™ showed marked reductions in overall pain (52%), pain upon limb manipulation (35%), and pain after physical exertion (40%). The active ingredients in FLEX CHOICE™ exert anti-inflammatory, immunomodulatory, and anti-osteoarthritic effects. ITPGS, in addition to being a bioenhancer, exerts its effects such as antioxidative and anti-inflammatory. No significant (P>0.01) change occurred in physical parameters, CBC, and serum biomarkers of liver and kidney functions. Findings revealed that FLEX CHOICE™ significantly ameliorated OA-associated pain, and it was well tolerated by dogs with moderate OA.

1674 Systemic and Genetic Toxicity Evaluation of Fermented *Citrus sunki* Hort. ex Tanaka Peel Extract

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Citrus sunki Hort. ex Tanaka peel is known as a rich source of various flavonoids, showing therapeutic activity on obesity, inflammation and allergy, and neuronal damage. Despite the increasing consumption of *C. sunki* peel products as herbal medicine and supplements due to the beneficial effects, their toxicity remained largely uncharacterized. In this study, we assessed the potential health risk of fermented *C. sunki* peel extract (FCPE) by conducting acute and 90-day repeated oral toxicity studies in Sprague-Dawley rats, and genotoxicity tests according to the OECD test guidelines. Single oral administration of FCPE did not cause abnormal clinical signs nor lethality in rats, establishing LD50 to be >2000 mg/kg BW. Administration of up to 2000 mg/kg BW FCPE for 90 days revealed no test substance-related toxicity as observed in analysis of body weight gain, food/water consumption, blood, serum biochemistry, weight and histopathology of organs, determining that NOAEL for FCPE is >2000 mg/kg BW. Additionally, FCPE showed no mutagenicity and clastogenicity at 5000 μg/plate in the reverse mutation and chromosome aberration assays, and 2000 mg/kg BW in *in vivo* micronucleus test. Collectively, our results demonstrated that FCPE lack systemic and genetic toxicity in the dose range we tested, suggesting its safety for human consumption.

1675 A Skin Sensitization Risk Assessment Framework for Evaluation of Metal Contamination in Personal Care Products

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Metal contaminants have been reported in a variety of personal care and cosmetic products. At sufficient exposures, metals can produce a variety of effects including skin sensitization. The goal of this analysis was to determine whether metals known to induce or elicit skin sensitization are found in commercially available hair care products and, if present, pose a health risk to consumers. Ten commercial hair care products were evaluated for the following metals: Ag, As, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Sn, and Zn via inductively coupled plasma mass spectrometry (ICPMS). A total of 6 metals (Be, Cd, Cu, Mn, Ni, and Zn) were identified between 4 of the products. Only 1 products were found to contain more than one of the metals. Of the metals identified, 1 has a cosmetic regulatory limit in the US (Zn), and 4 have cosmetic regulatory limits in the EU (Be, Cd, Ni, Zn). A survey of on-market consumer products revealed that 3 of the metals detected (Cu, Zn, Mn) were listed as ingredients in more than 4,000 products. Three of the detected metals (Be, Cu, Ni) were identified to have skin sensitization potential according to the ECHA and SkinSens databases and were evaluated further via a quantitative risk assessment. Daily consumer exposure levels to metals in hair care products were estimated using the amount of product applied per application, number of applications per day, metal concentrations in the products, a retention factor, and body surface area values. Estimated consumer exposure levels (mean and 95th percentile) were compared with metal-specific no expected sensitization induction levels or no observed effect levels in humans. Margins of safety (MOSs) were determined for products containing Be, Cu, and Ni. Similar methodology was used to calculate daily exposure to Be and Cu in shower water, assuming that the shower water contained the EPA drinking water standard concentrations for Be and Cu. All calculated MOSs were >1 for hair care products (MOS range: 4,776-47,619) and shower water (MOS range: 88-216,535). Interestingly, exposure to Cu in shower water alone was higher than exposure to Cu detected in the products evaluated. These results show metal contamination, at the levels detected, in the hair care products tested do not pose a skin sensitization induction concern.

1676 Safety Assessment of Acacia Seyal Gum for Use in Mascaras

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Acacia gums are edible ingredients used in foods and cosmetics. Acacia Seyal Gum (CAS# 9000-01-5) is an ingredient of interest for use in cosmetics (eye products/mascara). A typical pre-market safety review of cosmetics includes: 1) a public literature and vendor information review and 2) a test battery to fill any data gaps. Public toxicology-related literature was limited for Acacia Seyal gum. The literature review indicated that the Cosmetic Ingredient Review panel (CIR) has evaluated similar ingredients (Acacia Senegal gum and Acacia Senegal gum extract) and declared them safe as used in cosmetics (up to 9% in mascaras). However, Acacia Seyal gum was not specifically included in this review. The literature review also indicated that there were similarities between the previously CIR-reviewed Acacia Senegal gum and Acacia Seyal gum in terms of chemical, biochemical and structural characteristics. Acacia Senegal and Acacia Seyal gums are hyperbranched polysaccharide biopolymers rich in arabinose and galactose, similar in global biochemical and structural properties and somewhat different in the macromolecular conformation. Both are structured into polyproline type II helices. Both ingredients have high molecular weights (> 10^5 Da) and, thus, negligible potential for dermal absorption. Data from the CIR review have been incorporated into weight of evidence review and contributed to substantiating the safety of this botanical ingredient. However, to further substantiate the safe use of this ingredient in mascaras up to 1% at the final formula level, a robust test battery was designed to include chemical characterization (elemental impurities analysis), microbiological characterization, *in vitro* eye irritation (BCOP/CAMVA), clinical skin irritation (48-Hour Patch Test), clinical skin sensitization (Human Repeat Insult Patch Test), *in vitro* phototoxicity (UV spectrum, 3T3 assay). Results indicated that this ingredient conformed with Shiseido standards for elemental impurities, was of appropriate microbiological formula quality (met USP 51 criteria), was not an eye irritant, not a skin irritant, not a skin sensitizer, and not phototoxic. Consequently, Acacia Seyal Gum is safe for consumer use in the tested mascara formula up to 1% maximum use level under normal use conditions.

1677 The Toxicological Profile of Reklemel: A Reduced Risk Nematicide

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Reklemel™ is the commercial name for fluazaindolizine (CAS # 1254304-22-7), a new crop protection active ingredient for control of parasitic nematodes in various vegetable and fruit crops. Reklemel belongs to the sulfonamide class of chemistry and acts through a mode of action distinct from other commercial nematicides. Currently, fumigant products requiring specialized handling and stewardship approaches are highly represented in this market segment; thus, there is a significant opportunity to bring forward new products that will reduce human risk both from a hazard and exposure perspective. During development, the mammalian toxicology and human risk profile of Reklemel was compared to that of six registered nematicide active ingredients. Reklemel has a favorable acute toxicity profile (Oral LD_{50} = 1187 mg/kg; Dermal LD_{50} <5000 mg/kg; Inhalation LD_{50} > 5.8 mg/L), is not a dermal irritant nor sensitizer and is a moderate eye irritant. This profile was more favorable relative to two competitors and similar to the other four. When comparing end-use formulations, the profile of the product containing Reklemel was more favorable when compared to four out of six competitor end-use products. Reklemel was non-genotoxic based on the outcome of *in vitro* and *in vivo* assays which was a similar profile to competitors. Reklemel was shown to be non-carcinogenic in rats and mice. This profile was more favorable compared to four of six competitors. In the rat two-generation study there were no effects on fertility or other reproductive parameters nor did Reklemel cause developmental toxicity in rats or rabbits. The other active ingredients were similarly not specific reproductive or developmental toxicants. When comparing toxicity endpoints derived from sub-chronic and chronic toxicity studies, Reklemel had higher NOAEL values compared to all competitors. In summary, Reklemel has a favorable toxicity and reduced risk profile relative to several existing nematicides.

1678 A 13-Week Rat Inhalation Study of Aerosols Generated from Two Flavor Mixtures

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Two nicotine-free flavor mixtures (one containing 10.76% w/w menthol flavors and the other containing 1.15% non-menthol flavors) and a carrier mixture (glycerin and propylene glycol [PG]) were evaluated via a 13-week rat inhalation study in general accordance with OECD TG 413. Male and female Sprague Dawley rats were randomly assigned to groups for exposure to 1, 3, or 5 mg/L of aerosols of each flavor mixture, 5 mg/L carrier, or filtered air for up to 6 hours per day on a 5-day per week basis for 13 weeks, followed by a 6-week recovery. In addition, a satellite group of male rats were exposed on a comparable regimen to evaluate micronuclei (MN) in bone marrow (mammalian erythrocyte micronucleus test; OECD 474) and potential DNA damage in liver, lung, and nasal tissue (mammalian alkaline Comet assay; OECD 489). Plasma PG, a marker of exposure, increased with the increased aerosol exposure in both males and females at both weeks 4 and 11. There were no flavor- or carrier-related effects on survival, clinical observations, ophthalmic findings, or respiratory physiology after 13 weeks of exposure. In addition, no flavor- or carrier-related alterations in hematology, coagulation, serum chemistry, urinalysis parameters, or bronchoalveolar lavage fluid chemistry values and cytology were observed. Non-adverse, minimally lower mean body weights were noted in males and females exposed to 5 mg/L menthol flavor as compared to the filtered air groups, which correlated to slightly lower food consumption. Thymus weights in females exposed to 3 and 5 mg/L menthol flavor were lower than the 1 mg/L group and marginally lower (not statistically significant) than the filtered air and carrier groups. The decreased thymus weights were considered non-adverse given the lack of correlating histologic observations and the reversibility suggested at the recovery necropsy and may have represented a secondary stress response. Non-degenerative histologic changes of minimal to mild severity grades were observed in various nasal levels of males and females in all flavor and carrier groups at both the terminal and recovery necropsies. Both flavor mixtures and carrier were negative for the induction of MN in bone marrow and DNA damage in liver, lung and nasal tissue. Based on these findings, the no-observed-adverse-effect-concentration (NOAEC) was established to be 5 mg/L (the high-dose level) for each flavor mixture and the carrier.

1679 Toxicological Evaluation of Acrylonitrile Butadiene Styrene (ABS) 3D Printer Emissions

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Fused filament fabrication 3D printing with acrylonitrile butadiene styrene (ABS) filament emits billions of particles and numerous volatile organic compounds (VOCs). This study sought to investigate the toxicity of ABS emissions from a 3D printer both *in vivo* and *in vitro*. For the *in vivo* studies, Sprague Dawley rats were exposed to real-time ABS printing emissions or air (control) for 4 h/day, 4 days/week for 1, 4, 8, 15, and 30 days. The average aerosolized particle concentration was 0.24 ± 0.09 mg/m^3, and the average median particle electric mobility diameter was 85 nm with an average geometric standard deviation of 1.6. Benzene was the predominant VOC released during printing. At 24 h after the last exposure, rats were assessed for pulmonary injury, inflammation, and oxidative stress as well as systemic and other organ toxicity. Results showed that among the measured cytokines in bronchoalveolar lavage, only IL-10 and IFN-γ at day 1 and 4, and IL-13 at day 30 of the exposure were increased when compared to the air-control. Moreover, neither pulmonary oxidative stress responses nor histopathological changes of the lungs were found among the exposed rats. There were no significant differences in serum cytokines levels or hematological indices, except for an increase in platelets and monocytes at day 15. Several serum biomarkers involved in liver damage were significantly higher at day 1 of the exposure. For the *in vitro* study, both particles and VOCs were collected into serum-free cell culture medium using an impinger sampler inside a chamber while printing for 1.5 h, followed by characterization of the physicochemical properties, as well as assessment of cytotoxicity, oxidative stress, and cytokine production in human small airway epithelial cells (SAEC). Results showed that particle numbers and VOC concentrations varied between print runs. The particle dose range was 1.42 x 10^6 - 4.72 x 10^6 particles/cm^2, and the average median hydrodynamic particle diameter was 168 nm with an average arithmetic standard deviation of 53. Styrene was the predominant VOC collected in the medium. Based on mixed model regression analyses, at 24 h post-exposure, ABS emissions in-

duced significant dose-dependent cytotoxicity, oxidative stress, and production of pro-inflammatory cytokines in SAEC. In conclusion, our *in vitro* studies indicated that the emissions from ABS 3D printing induced toxicological effects, which were not substantiated by the *in vivo* studies with the current low exposure concentrations. Thus, more *in vivo* studies with higher dose-response are needed to verify the *in vitro* findings.

1680 Retrospective Review of Safety Data for the Vehicle Sulfobutyl-ether-β-cyclodextrin (SBE-β-CD) in the Nonclinical Studies

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Sulfobutyl-ether-β-cyclodextrin (SBE-β-CD), also known as Captisol. SBE-β-CD is used in several commercial drugs and also as a common vehicle in non-clinical studies. Sixteen studies involved SBE-β-CD in mice, rats, dogs and monkeys were reviewed and the data analyzed to further evaluate the safety of Captisol when used as part of the vehicle formulation. In eleven studies (5 rat studies; 4 dog studies; and 2 monkey studies) 198 mg/kg/day to 1000 mg/kg/day SBE-β-CD was dosed for 28 consecutive days. No vehicle related adverse effects were noted up to 1000 mg/kg/day oral dose in rats, dogs and monkeys in all above studies. Base on the study results the NOEL of SBE-β-CD for oral dose in rats, dogs and monkey was considered as 1000 mg/kg/day. Mice were more sensitive to the oral exposure to the SBE-β-CD in three exploratory studies. The duration was from 5 days to 28 days and the dosages were from 850mg/kg/day to 1960 mg/kg/day. Increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) were noted in these studies along with histopathology changes as hepatocellular necrosis accompanied by neutrophilic infiltration. In two GLP studies when SBE-β-CD (from 960 to 4800mg/kg/day) was administered by intravenous in rats and dogs, vacuolations and foamy macrophases in various tissues or organs were noted in the histopathology. In the dosing phase, histopathologic findings associated with the SBE-β-CD included vacuolation of the renal tubular epithelial cells and epididymis along with an infiltrate of foamy macrophages in a variety of lymphoid organs, spleen, liver, lungs, cecum, heart, urinary bladder and uterus. The histopathological changes in morphology due to SBE-β-CD were not associated with any cellular degeneration and/or necrosis. Histopathologic findings associated with SBE-β-CD in the heart, cecum, lungs, spleen urinary bladder, kidney, epididymis, mandibular lymph node and mesenteric lymph node were revisable. There was no recovery of the histopathologic findings in the liver and uterus. In conclusion the toxicity of SBE-β-CD is dependent on the species and route of administration in the non-clinical studies. Oral dose was safe for most of the species such as rats, dogs and monkey but except for mice which could induce liver related changes in both clinical pathology and histopathology data. Intravenous infusion administration resulted vacuolations and foamy macrophages in various tissues or organs but not associated with any cellular degeneration and/or necrosis.

1681 Hazard Potential of Perfume Products Assessed by a Combination of *In Vitro* Methods and Chemical Analysis

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Alternative *in vitro* methods and studies on human volunteers are the only hazard assessment approaches available, as animal testing is prohibited in the EU for cosmetic ingredients and final products. In our study we tested 10 samples of deodorants, EDT and EDP and combined results from biassays, suitable for detection of cytotoxicity (ISO EN 10993-5), skin sensitization *in chemico* (OECD TG 442C) and *in vitro* (OECD TG 442D), genotoxicity (Comet assay on 3T3 Balb/c fibroblasts) and endocrine activity (YES/YAS assay; Xenometrix®). Samples 1, 2, 3, 5 exhibited the highest cytotoxicity. The skin sensitization was identified by a method *in chemico* for samples 5, 6, 7, 8 and a method *in vitro* at 12.5 µg/ml for sample 6, resp. at 100 µg/ml for samples 7, 8, 9. Comet assay detected no genotoxicity at 250 µg/ml, however, a concentration dependent DNA fragmentation was observed (samples 6, 8, 9, 10). Samples 1, 2, 5, 6, 7, 8, 9, 10 exhibited endocrine activity. 24 allergens (INCI names: D-limonene, linalool, benzyl alcohol, citronellol, methyl 2-octynoate, geraniol, citral, hydroxycitronellal, cinnamal, anise alcohol, cinnamyl alcohol, eugenol, alpha-isomethyl ionone, isoeugenol, butylphenyl methylpropional, coumarin, farnesol, amyl cinnamal, hydroxyisohexyl 3-cyclohexene carboxaldehyde, amylcinnamyl alcohol, hexyl cinnamal, benzyl benzoate, benzyl salicylate, benzyl cinnamate) were determined by GC/MS. Although the hazard of individual components is known, the hazard of the final mixture may be variable, depending on either total load of ingredients or the content of specific active substances. Combination of bioassays and chemical analysis seems to be promising for risk assessment of cosmetics. *The work was supported from ERDF/ESF project "International competitiveness of NIPH in research, development and education in alternative toxicological methods" (No. CZ.02.1.01/0.0/0.0/16_019/0000860).*

1682 Comparative Subchronic Inhalation Toxicity 2-Pentanoneoxime

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Oximes are formed by the reaction of hydroxylamine with the carbonyl group of either an aldehyde or a ketone. In the body, the reaction reverses and the hydroxylamine is released. Hydroxylamine then causes hemolysis of the red blood cells resulting in the release of hemoglobin and an increase in platelets. As the liver and spleen filter the hemolyzed red blood cells, this can cause an increase in cell turnover. The carcinogenic potential of 2-pentanoneoxime (2-PO) was evaluated in a genomics study following a 90-Day exposure study. Groups of 10 male and 10 female Sprague Dawley rats were exposed to levels of 0 (control), 50, 150 and 300 ppm of 2-PO 6 hours/day, 5 days/week for 13 weeks and then sacrificed. Two recovery groups of 5 male and 5 female rats were also included. They were exposed to 0 and 300 ppm of 2-PO for 13 weeks and then held for a 4 week recovery period. 2-PO was metabolized to methylpropylketone (MPK). Clearance from the body was rapid. At 6 hours post exposure, neither 2-PO nor MPK was seen in the blood. Hemoglobin levels showed a slight decrease (4.4%), reticulocytes showed a increase (31,3%) and platelets showed an increase (27.9%) in the high exposure level males, no differences were seen in females. In the rats sacrificed at the end of the 13 week exposure period, increases were seen in the relative kidney weights in both male and female rats exposed to 300 ppm. Also relative spleen weights were increased in female rats exposed to 300 ppm. All organ weight effects were reversible following the 4 week recovery period. No treatment related histopathological alterations were seen in any of the organs evaluated. Since the changes were minimal, only seen in one sex and fully reversible in the rats held for the 4 week recovery period, they were considered to be non-adverse. It was therefore concluded that the No Adverse Effect Level for a 13-week exposure to 2-PO was 300 ppm. In the 2-PO exposed rats, there were no observations of treatment related effects during necropsy. In the microscopic examination of tissues from the 300 ppm exposure level group, the only observation that may have been related to the exposure was a reversable increased frequency of mild mononuclear inflammation of the liver in the female rats. This would not have caused a significant increase in apoptosis. 2-PO was evaluated in a genomics assay by a highly competent laboratory and found to not activate the genes associated with liver cancer. This assay has been used for over a decade and found to accurately predict carcinogenic potential. The results from this assay should be trusted for the safety evaluation of 2-PO.

1683 Comparing Cytokine Data to In-Life Parameters on Nonhuman Primates in Nonclinical Toxicology Studies

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Cytokines are important immunoregulatory proteins that have gained attention in safety assessment associated with innate or adaptive immune responses. Interpreting cytokine data comes with challenges due to the variable nature of their stimuli and responses. Contributing factors to the variability in cytokine expression include species-specific reactions, individual variations, dose-response relationships, and unanticipated immunotoxicity. For these reasons, cytokine measurements should not be used as standalone biomarkers for immunotoxicity assessment. However, in conjunction with additional parameters such as clinical observations, body weights, and clinical pathology data, cytokine interpretation can be used to provide more definitive assessments in nonclinical safety studies. In several case studies, cytokines were evaluated for a dose response relationship. Multiplex platforms such as Luminex or MSD® were used to determine cytokine levels in non-human primates including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12/IL-23p40, MCP-1, IFN-γ, and TNF-α. In several instances, measurable levels of IL-6 or IL-12 correlated with clinical observations of bruising, injury, or abnormal feces, not necessarily considered test article-related. Elevated TNF- α and IL-6, pro-inflammatory cytokines, were detected in animals observed as dehydrated with elevated BUN, creatine, and decreased electrolytes. In cases of test article-related effects, animals becoming moribund also had elevated TNF-α and IL-6 levels. Increased levels of MCP-1, a monocyte chemotactic factor, was observed in one study with an animal with petechial bruising, and another study with a cohort with test-article associated renal failure, characterized by hypoproteinemia, azotemia, and hyperkalemia. In conclusion, cytokines are useful markers

when assessing potential toxicity when evaluated with other measurements. Clinical observations, body weights, and clinical pathology parameters should also be considered in addition to the test article-related effects.

1684 Neurobehavioral Assessments of Juvenile Male and Female Wistar Han IGS Rats, Hsd:Sprague Dawley SD Rats, and CD-1 IGS Mice

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The objective of these studies was to generate juvenile neurobehavioral historical control data in Wistar Han IGS (WH) rats, evaluate prepulse inhibition (PPI) testing protocol in Hsd:Sprague Dawley (SD) rats and CD-1 IGS mice, and compare these to positive control-treated animals. Motor activity evaluations were conducted on WH rats between Postnatal Day (PND) 70 - 80 and between PND 120 - 130, after dosing with 0 or 2.0 mg/kg amphetamine. Learning and memory were evaluated using passive avoidance, conducted on WH rats between PND 80 - 90 and between PND 125 - 135, after dosing with 0 or 2.0 mg/kg scopolamine, and Morris Water Maze testing with WH rats between PND 75 - 85 and between PND 120 - 130, after dosing with 0 or 2.0 mg/kg scopolamine. Auditory startle habituation was conducted on WH rats between PND 70 - 80 and between PND 120 - 130, after dosing with 0 or 1.0 and 0 or 0.3 mg/kg MK-801, respectively. Auditory startle habituation with PPI was conducted on SD rats and CD-1 mice between PND 60 - 70, after dosing with 0, 0.3 mg/kg MK-801 or 1.0 mg/kg Midazolam. The vehicle and control articles were administered on neurobehavioral testing days via intraperitoneal injection. All control articles were administered at a dose volume of 1.0 mL/kg. The saline -treated animals received the vehicle in the same manner, at the same volume, and within the same time frame prior to each neurobehavioral test. Amphetamine increased motor activity parameters in both sexes of WH rats at both ages. When assessing learning and memory, scopolamine altered the parameters tested in the Morris water maze in WH rats to a larger degree than compared to passive avoidance parameters in both male and female and at both ages. MK-801 reduced acoustic startle habituation in the male WH rats at PND 70 - 80, females at both ages, and to a lesser extent in males at PND 120 - 130. In male and female SD rats, PPI was reduced by MK-801 and Midazolam decreased the amplitude of the peak response in male SD rats only. In male and female CD-1 mice, MK-801 did not affect PPI and increased the mean peak response. Midazolam did not affect males or females PPI or mean peak responses. These results demonstrate the ability to detect the effects of chemicals on neurobehavioral parameters and the differences in the responses of the test animals based on species, age, and sex and provided historical background data for juvenile neurobehavioral studies.

1685 Understanding the Differences between *In Vitro* versus *In Vivo* Results: The Role of Kinetics and Tissue Dose Metrics

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With the increasing emphasis on moving from animal-based toxicity testing to *in vitro* assays, there is also an increasing need to determine the characteristics that make *in vitro* screening assays predictive and reliable. In this case study, we determined that differences between positive *in vitro* and negative *in vivo* results were due to rapid *in vivo* clearance of the test compound. The test compound, BAG8, is a benzoxaborole broad-spectrum fungicide that was tested for *in vitro* mutagenic activity (Ames assay) and *in vitro* clastogenic potential (micronucleus). Whereas BAG8 was negative in an Ames assay, it was negative at all doses without metabolic activation and positive with metabolic activation in an *in vitro* micronucleus screening study in a dose responsive manner below cytotoxic concentrations. Therefore, BAG8 was evaluated for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by counting micronuclei in reticulocytes in rat peripheral blood. No significant increase in the incidence of micronuclei was observed in BAG8 exposed animals. In a separate study, BAG8 demonstrated rapid clearance *in vivo*, with an oral Tmax of 15-30 minutes. Therefore, the positive *in vitro* finding with metabolic activation may be attributable to one or more BAG8 metabolites which may be short-lived *in vivo*. *In vitro* studies are valuable, inexpensive, animal-sparing test systems, which may be leveraged to focus *in vivo* research such as verification of positive results, kinetics, metabolism and tissue exposure levels.

1686 Cytoplasmic Vacuolation and Tapetal Changes Induced by a Novel Analgesic Agent in Beagle Dogs

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Drug-induced unique cytoplasmic vacuolation was found in the subchronic oral toxicity study of 4-dimethylamino-1-{3-(1-methyl-1H-imidazole-2-yl)propanoyl}piperidine (DMIP), a potential therapeutic agent for neuropathic pain, in beagle dogs. In the first study, DMIP was administered at a dose of 250, 500, or 1,000 mg/kg/day once daily for 14 days. Discoloration of tapetum lucidum accompanied by tapetal swelling was observed at ≥ 250 mg/kg/day. The tapetal swelling was correlated to the light microscopic observation of cytoplasmic vacuolation in tapetal cells, and similar vacuolation was observed in several other tissues, including the coronary artery and aortal arch, in a dose-dependent manner. Immunohistochemistry for lysosomal-associated membrane protein 2 indicated that the vacuoles were enlarged lysosomes. However, the nature of these vacuoles was different from that of phospholipidosis because no lamellar bodies were observed. In the second study, DMIP was administered at a dose of 10, 50, or 250 mg/kg/day once daily for 14 days followed by a 14-day recovery period. Tapetal changes and systemic vacuolation were not observed at ≤ 50 mg/kg/day, and vacuolation observed at 250 mg/kg/day was reversible. A few reports have described the enlargement of lysosomes not attributable to phospholipid accumulation. Our findings provide further information about the toxicological implications of drug-induced lysosomal swelling.

1687 Establishment of a 3D Human NASH Model for High-Throughput Drug Efficacy Testing

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Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of lipids within the liver and can progress from simple fatty liver to more serious chronic diseases ranging from nonalcoholic steatohepatitis (NASH) to liver fibrosis, and cirrhosis. Currently, there are no approved, safe therapies for NASH and the development of novel therapeutics is impeded by the lack of predictive *in vitro* models to help decipher the complex mechanisms underlying the disease progression. The aim of this study was to develop a 3D human NASH model containing primary hepatocytes, Kupffer cells, endothelial cells, and hepatic stellate cells for high-throughput compatible compound efficacy testing. The microtissues models were cultured in high-throughput 96- and 384-well plates, enabling reproducible tests and screening. Using a defined cocktail of free fatty acids and LPS in medium containing high levels of sugars, we sought to recapitulate NASH pathogenesis *in vitro*. For drug efficacy testing, we simultaneously treated the 3D microtissue model with clinical NASH drug candidates (Firsocostat and Selonsertib), then measured endpoints related to the hallmarks of the disease. Upon treatment with NASH stimuli, liver microtissues displayed key pathophysiological characteristics of NASH, such as increased tissue triglyceride levels as well as increased secretion of inflammatory and pro-fibrotic markers. Consistent with clinical trial data, results showed a decrease of triglyceride levels and secretion of inflammatory markers, respectively. Additionally, we demonstrated that the TGFRβ1 inhibitor ALK5 inhibitor dose-dependently decreased the release of pro-collagen type I and deposition of fibril collagens. In summary, this 3D human NASH model for preclinical, high-throughput compound screening, represents a promising approach for early selection of the most effective and least toxic drug candidates for advancing in the clinical drug development pipeline.

1688 Evaluation of Whole Blood T Cell Receptor Codon Usage as Biomarker for Rorg-Related Thymic Lymphoma

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RORγT is a sequence variant of the nuclear hormone receptor RORγ that is expressed exclusively in the thymus. RORγT $^{-/-}$ mice develop thymic lymphoma with high penetrance and short latency, but the pathogenesis is not well understood. We sought to determine whether T cell receptor alpha (TCRα) variable region rearrangement correlated with the development of thymic lymphoma. TCRα variable region usage was evaluated in blood and in thymi of constitutive RORγT knockout (KO) and inducible RORγ KO mice by quantitative PCR analysis. In RORγT $^{-/-}$ mice, the 5′ TCRα variable regions 1 and 2 (Trav1

and v2) were undetectable, whereas the 3′ region Trav21 was markedly elevated in thymus and blood samples relative to wild type (WT) littermates, indicative of a less mature genotype of TCRα. In inducible KO mice, the skewed TCRα codon usage in thymus correlated with similar changes in blood and with development of lymphoma. RORγT $^{+/-}$mice, which do not develop lymphomas, showed reduced, but clearly detectable Trav1 and Trav2 and a slight increase in Trav21. Our results show that genetic RORγT loss in mice causes strong perturbations of T cell receptor codon usage (TCRα phenotype), as well as development of thymic T cell lymphomas, whereas mice with an incomplete knockdown had an intermediate TCRα phenotype and no development of lymphoma. These results also indicate that peripheral blood can be used to monitor for a level of RORγT-related change in TCRα usage which is associated with a risk of developing thymic lymphomas.

1689 Validating Proposed Improvements to Phototoxicity Expert Alerts against Proprietary Data

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Development of expert alerts for toxicological endpoints is a time-consuming process, especially if changes are subsequently requested to these alerts based on proprietary data which for legal reasons cannot be shared with the original implementer even under strict confidence. We present a method for the acceleration of this process, analogous with the student-teacher model[1] used for statistical methods in similar situations. In order to demonstrate this method, the endpoint of phototoxicity was chosen. This has become of increasing regulatory concern for pharmaceuticals, as evidenced by the ICH S10 guidelines[2]. A dataset of 400 publically-available structures with phototoxicity data was collated, and, working from the knowledge base of expert alerts contained within Derek Nexus 2018.1 (Lhasa Limited, Leeds, UK), a number of alerts were created or modified on the basis thereof. These modifications improved the performance of the alerts against this public dataset from an F1-score of 75% to 83% and Matthews correlation coefficient from 45% to 49%, including an increase in sensitivity from 63% to 77%. A pre-release version of the improved knowledge base was provided to three pharmaceutical companies who evaluated performance against their proprietary datasets. Specific recommendations for modifications to structure-activity relationships were returned and collated. Information from confidential structures was therefore absorbed without these structures being disclosed, improving predictive performance against both public and proprietary chemical space prior to the main release of the knowledge base to a wider set of users. This poster serves not only to demonstrate the method used but also to highlight the toxicophores considered in this process. *[1] Papernot et al, arXiv:1610.05755v4 [2] ICH Guideline S10, available at https://database.ich.org/sites/default/files/S10_Guideline.pdf.*

1690 Novel 3D Liver Microtissue Reactivity Assay Reveals Causal Link between Glutathione Status and Cytotoxicity

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Formation of reactive drug metabolites and reactive oxygen species (ROS) is linked to a high risk for the development of Drug-Induced Liver Injury (DILI). Reactive drug metabolites can bind covalently to proteins, inactivating important cellular functions or leading to hapten formation triggering immune reactions. The glutathione (GSH) system in the liver plays an important detoxification role by scavenging reactive metabolites. The role of reactive metabolites and ROS formation in cytotoxicity has not been fully characterized. The model toxicant acetaminophen (APAP) forms reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is detoxified by the GSH system. APAP treatment, in the absence of the GSH depleting agent, L-buthionine-sulfoximine (BSO), leads to concentration-dependent decrease of cellular ATP levels, without increase of the cytotoxicity marker LDH. 3D Human liver microtissues (LiMTs) consisting of primary hepatocytes, Kupffer cells, and liver endothelial cells offer high translational value for de-risking drug development due to their longevity, stable metabolic competence and preserved functionality. Microtissues are cultured in high-throughput (HT) 96- and 384-well plates, enabling reproducible tests and screening. The aim of this study was to demonstrate the feasibility of causality assays by the increased sensitization of microtissues to APAP in the presence of BSO treatment. We determined experimental conditions under which BSO reduced cellular GSH levels but did not affect cellular viability allowing for longer compound exposure. Changes in APAP cytotoxicity profiles in the presence of BSO were monitored with a set of parameters, such as a shift of the ATP IC_{50} and LDH leakage indicating membrane damage. Additionally, we showed that release of LDH correlates with release of clinically relevant AST in our *in vitro* model, providing an HT-compatible alternative for cytotoxicity monitoring. These results indicate a causal link between cellular GSH levels and cytotoxicity in 3D human liver microtissues. The liver microtissues in the HT format, combined with the cytotoxicity assays (ATP and LDH), provide a basis for screening compound libraries in early phase drug development to eliminate the most toxic compounds, which might trigger DILI.

1692 Development of an Automated Target Knowledge Review Application to Accelerate Target Safety Evaluations

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Pfizer's Drug Safety Research and Development (DSRD) division has been systematically evaluating information on drug targets for the last 15 years to drive a deeper understanding of safety risks at all stages of development. Best practice workflows contributing to these Target Knowledge Reviews (TKRs) were honed over time into a well-defined process involving manual curation of data and information on targets from a number of public and private sources and databases. Historically a manual, time consuming process, DSRD has worked recently to automate as much of the process as possible to make it more efficient and standardize the TKR content. A MySQL database containing target-centric data from external (e.g. Ensembl, NCBI, Uniprot, MGI, GTEX, HPA, ChEMBL) and internal (e.g. TOE/Human Genetics, Tissue Map mRNA expression, Linguamatics I2E) sources and application code (R, Shiny) were deployed as part of the automation solution. The application provides a simple user interface (UI) for target search and selection, followed by either web-based tab display of target information or generation and download (~ 20 seconds) of the Automated TKR (AutoTKR) report. The architecture of the application (database, code, UI) allows for rapid and seamless integration of new data and functionalities as they are developed. AutoTKR uptake in DSRD has been broad, contributing to virtually all new TKRs in the portfolio.

1693 Target Safety Assessments: Evaluation of the Toxicological Risk of Targeting Plasmepsins in the Treatment of Malaria

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Plasmepsins are aspartic proteases that are important antimalarial drug targets due to their specificity to the malaria parasite and to their vital roles such as mediators of parasite egress and invasion of red blood cells (RBC). Focusing on parasite-specific targets for which no human host homologue exists reduces the chance of drug toxicity. However, adequate selectivity for plasmepsins in Plasmodium over related mammalian aspartic proteases is critical. To fully investigate the potential for host toxicity we conducted an *in silico* target safety review to understand the risks associated with host aspartic protease inhibition. We studied mammalian aspartic proteinases including pepsin, cathepsins D and E (Cap D/E), β secretase 1/2 and renin. Cap D/E appear of most toxicological relevance as Cap D is a ubiquitous lysosomal enzyme present in most cell types and Cap E is found in epithelial cells lining the gut (exposed to orally administered drugs) and in RBCs (the clinically significant site of malarial infection). Based on mammalian aspartic protease physiology and adverse drug reactions (ADRs) to FDA-approved HIV aspartic protease inhibitors, we predicted several potential toxicities including β-cell and congenital dysfunction, hypotension, hypopigmentation, lipideamia, increased infection risk and respiratory, renal, gastrointestinal, dermatological and other epithelial tissue toxicities. This target review indicated that ADRs to the HIV treatments are likely a result of host aspartic protease inhibition due to a lack of specificity for the HIV protease and that plasmepsins are much more closely related to human Cap D than to HIV proteinase. Plasmepsin inhibition presents an opportunity to specifically target Plasmodium as an effective antimalarial treatment. However, adequate Plasmodium selectivity is critical. Plasmepsin inhibitors should be assayed for inhibitory activity against the main human aspartic proteases, with priority given to cathepsin D and E. As the targets are plasmodium specific enzymes, a safety assessment plan would depend on which (if any) mammalian aspartic proteases are inhibited by the lead compound(s). It would be useful to conduct an investigative rodent study early in drug development to identify whether the risks we have identified would occur *in vivo* with a plasmepsin inhibitor.

1694 Effects of Diminazene Aceturate and Ivermectin on Semen and Serum Parameters of the Red Sokoto Buck

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The effects of diminazene aceturate and ivermectin on semen parameters, serum testosterone and serum follicle stimulating hormone levels of the red Sokoto buck was investigated. Twenty seven red Sokoto bucks, at the age of 2 years and weighing between 32 - 34kg were used. After administration of the drugs, semen and sera samples were collected 1, 24, 72, and 192 hours for analysis. The parameters studied *viz* semen volume, percentage motility of sperm, sperm concentration, live sperm percentage, semen glucose level, serum testosterone and serum follicle stimulating hormone were found to decrease significantly ($P<0.05$) when compared with the control group throughout the collection period. However, the drugs did not affect the live sperm percentage and ivermectin did not affect semen glucose level. A relationship was established between spermatological characteristics and serum testosterone and follicle stimulating hormone levels. These findings indicate that the drugs investigated in this study decreased semen parameters and serum testosterone and follicle stimulating hormone. It was concluded that diminazene aceturate and ivermectin should be used cautiously in red Sokoto bucks meant for breeding due to the deleterious effects they were observed to have on fertility parameters.

1695 Uncoupling NRF2 Activation from Induction of Hyperkeratosis: Keap1 Inhibitors with Different Binding Modes Show Discriminating Histopathological Profile

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Oxidative stress is a key driver for many chronic diseases and induction of a cytoprotective, anti-oxidant program via NRF2 activation is thus therapeutically attractive. KEAP1 KO mice are postnatally lethal due to malnutrition, correlating with NRF2-mediated hyperkeratosis in oesophagus and forestomach. Hyperkeratosis is associated with epidermal thickening and dysregulation of differentiation-specific keratins. Here we set out to investigate whether pharmacological inhibition of KEAP1-mediated degradation of NRF2 via different mode of actions, using a protein-protein interaction inhibitor (PSTC) and an electrophile (Bardoxolone), induces similar downstream molecular events and physiological effects. Female C57Bl/6 mice were orally dosed at 10, 30 and 100mg/kg/day for 4 days and key target organs were sampled for assessment of gene expression and histopathology. Target engagement was confirmed with both compounds as monitored by activation of NRF2-driven genes *Hmox1, Srxn1, Nqo1*. However, only Bardoxolone induced hyperkeratosis and Keratin 6 (*Krt6a*), a marker for proliferating keratinocytes, in oesophagus and forestomach whereas PSTC did not. Thus, this study demonstrates the potential for pharmacological NRF2 activation by KEAP1 inhibition without inducing hyperkeratosis and identifies that Keratin 6 may be used as a novel exploratory safety biomarker.

1696 Computational Approach for Prediction of Drug Target Safety Assessment

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A review of industry drug discovery and development projects showed that most important reason for drug failure in recent decades is unacceptable off-target and on-target related safety explaining more than 50% of all project closures. In order to help assessing target safety we generated a comprehensive database and software, with computable internally curated and various publicly available data on proteins, biologically active chemicals, their interactions, pathways and pathologies. We catalogued 4 million references supporting each database entry, with hyperlinked interactions to appropriate PubMed article as support while proteins and chemicals are hyperlinked to EntrezProtein, PubChem of the other appropriate public database. In order to support data integration and capture different levels of histopathological observations, we developed toxicology ontology with over 2500 toxicity endpoints. We validated the system by exploring androgen receptor (AR), a high data density target with known safety liabilities. Mapping AR onto GO function within the system promptly identified AR biological function in sex differentiation and male gonad development including prostate gland development. Mapping AR together with its protein interaction network onto Diseases and Organ and tissue pathologies, associated AR with several pathologies and toxicities related to development including Swayer syndrome and Y chromosome deletion. Furthermore, the system associated AR with other pathologies on cellular organ and organ system level related to cardiac system (heart fibrosis, cardiac hypertrophy) nervous system (neuron degeneration), male and female reproductive system (testis atrophy, oligospermia, prostate toxicity, mammary gland hyperplasia and uterus atrophy), hepatic system and endocrine system (adrenal gland hypertrophy). Analyzing AR together with its chemical interaction partners (agonists and antagonists) identified AR role in Prostate neoplasia and Prostate carcinoma toxicity that corresponds to AR antagonist therapeutic application. On this example we showed that our tool can contribute to a better understanding of therapeutic potential of a target inhibition and quick target toxicological assessment.

1697 *In Vitro* Cytotoxicity Assays: Development of a Cell-Based Model to Screen the Potential Toxicity of Chemical Compounds

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Cytotoxicity assay is useful for screening toxicity providing predictive evidence of compound safety. In this study, we assessed three cytotoxicity assays and enzyme activity of hepatocytes to detect the cytotoxic event. Human hepatoma cell line, namely HepG2 cells, were seeded at the density of 1.5 x 10^4 cells/well on 96-well cell culture plates and were incubated at 37°C in a 5% CO_2 humidified incubator. After 24 hours post-seeding, cells were treated with acetaminophen (1, 3, 10, 30 mM), amiodarone HCl (10, 17.8, 31.7, 56.4, 100 uM), etoposide (100, 178, 317, 564, 1000 uM), orphenadrine hydrochloride (30, 53, 95, 169, 300 uM), and lovastatin (30, 53, 95, 169, 300 uM) for 24 hours. Following to the drugs treated, cytotoxicity was determined with the 4-[3(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1), the methyl tetrazolium (MTT), and neutral red uptake (NRU) assays and alanine transaminase (ALT), aspartate transaminase (AST), and lactate dehydrogenase (LDH) enzyme activity. Cytotoxicity assays and enzyme activity were observed to depend on each drug concentration except acetaminophen. No acetaminophen-induced changes in enzyme activity were observed, whereas the cytotoxicity assays were detected depending on concentration after 24 h. It was shown that the MTT or NRU assays were more sensitive at lower concentrations in cytotoxicity assays. In conclusion, cytotoxicity assays have different sensitivity depending on the mechanism of cellular damage. The results demonstrate that the application of cytotoxicity assays enables efficient screening, and further, they suggest that certain parameters could serve as sensitive methods for predicting the cytotoxicity potential of chemical compounds.

1698 Safety Evaluation of High Concentration of Live Bacteria on Models with Compromised Physiological Barriers

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The frontier between commensals and pathogenic bacteria is not straightforward. It has been well acknowledged that some bacteria normally considered as commensals, can become pathogenic when they escape their original niche and start to colonize deeper tissues. Inflamed and compromised physiological barriers may facilitate the change in characteristic of harmless bacteria. The safety of high concentration of Lacto*Bacillus* formulations was evaluated in animal models where physiological barriers were altered, namely a chronic dextran sodium sulfate (DSS) induced colitis and oxazolone-induced atopic dermatitis murine models. In the colitis model, mice received DSS for 3 cycles of 5 days separated by 7 days. Bacterial dosing, individually or as a combination of the three strains, was performed daily. Stool and body weight were evaluated and a total disease activity index (DAI) was calculated. Colons were collected for histopathology evaluation. In the atopic dermatitis, formulations were topically applied for 21 days. Terminal blood samples for clinical pathology evaluation as well as histopathological evaluation of various tissues were conducted. In the AD model, the treatments were well tolerated. There was no treatment related changes in serum biochemistry or hematology parameters. Histopathological evaluation revealed no findings in the brain, liver, lungs, kidneys, spleen, muscles and heart of treated mice. In the colitis model, the treatments were also well tolerated with no sign of systemic inflammation or infection. Treatment with live bacteria markedly reduced local inflammation and progression of colitis in colon samples, confirming ef-

ficacy of the treatment. There were no microscopic changes in the spleen and liver that were attributed to treatment with test items. No abscess formation, bacterial colonies, and extensive inflammation or necrosis were observed in any treatment group suggesting significant translocation of bacteria was not associated with administration of the test items. Our data clearly indicate that treatment of mice with our combination of strains on colitis or on dermatitis compromised barriers produces no significant safety concern according to parameters evaluated. These bacterial strains present an alternative approach in treatment of colitis.

PS 1699 Simple High-Throughput Workflows for Assessing Drug-Induced Mitochondrial Dysfunction Using Oxygen Consumption Measurements

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Recent years have seen a growing appreciation of the importance of mitochondria as sites for off-target drug effects, contributing to safety-related attrition and post-market withdrawals. Robust assay workflows are therefore required to enable convenient, high-throughput identification of direct, specific mitochondrial impairment. Additionally, as some toxicity is mediated by bioactivation or bioaccumulation, longer-term drug exposures need to be facilitated, ideally with the capacity to delineate cytotoxicity from perturbed metabolism. Here we describe the development and evaluation of two discrete workflows that address these specific challenges by measuring the impact of drug-treatment using the MitoXpress Xtra Oxygen Consumption Assay. The first workflow describes a 384-well assay for acute impairment of mitochondrial function using HepG2 cells in suspension, facilitating a streamlined workflow that minimizes cell preparation time, reagent addition, and washing steps. Assay performance is evaluated using a compound panel with known mitochondrial liabilities, including Ketoconazole, Tamoxifen, Nefazodone, and Flutamide. Z' values of > 0.6 are consistently achieved and observed dose responses are consistent with previously reported data, highlighting the utility of this approach in the high-throughput detection of direct mitochondrial liabilities. To enable longer-term drug exposures, a second complementary workflow was developed and evaluated using adherent HepG2 cells in 96-well plates treated with drugs >24h prior to measurement. As non-specific cytotoxicity can confound interpretation of metabolic impairment after longer treatment times, this workflow comprises a multiplexed oxygen consumption/cytotoxicity assessment to better delineate metabolic responses after extended treatment times. Dose-dependent effects of model drugs were again consistent with reported data, while differential metabolic and cytotoxic responses are seen to distinguish non-specific cytotoxicity from specific mitochondrial impairment. Together these data illustrate the utility of oxygen consumption-based assessments of drug-induced mitochondrial dysfunction, enabling both rapid, simple, high-throughput screening for acute mitochondrial toxicants, and longer-term multiplexed assessment of drug-induced metabolic perturbation.

PS 1700 Small-Scale Panel Comprising Diverse Gene Family Targets to Evaluate Compound Promiscuity

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Despite the recent advances in the life sciences and the remarkable investment in drug discovery research, the success rate of small-molecule drug development remains low. Safety is the second most influential factor of drug attrition in clinical studies; thus, the selection of compounds with fewer toxicity concerns is crucial to increase the success rate of drug discovery. Compounds that promiscuously bind to multiple targets are likely to cause unexpected pharmacological activity that may lead to adverse effects. Therefore, avoiding such compounds during early research stages would contribute to identifying compounds with a higher chance of success in the clinic. To evaluate the interaction profile against a wide variety of targets, we constructed a small-scale promiscuity panel (PP) consisting of eight targets (ROCK1, PDE4D2, GR, PPARγ, 5-HT_{2B}, adenosine A_3, M1, and $GABA_A$) that were selected from diverse gene families. The validity of this panel was confirmed by comparison with the promiscuity index evaluated from larger-scale panels. Analysis of data from the PP revealed that both lipophilicity and basicity are likely to increase promiscuity, while the molecular weight does not significantly contribute. Additionally, the promiscuity assessed using our PP correlated with the occurrence of both *in vitro* cytotoxicity and *in vivo* toxicity, suggesting that the PP is useful to identify compounds with fewer toxicity concerns. In summary, this small-scale and cost-effective PP can contribute to the identification of safer compounds that would lead to a reduction in drug attrition due to safety issues. *(Reprinted with permission from Chemical Research in Toxicology. Copyright 2019, American Chemical Society).*

PS 1701 Pro-Arrhythmia Risk Assessment and Late Sustained Sodium Current (INa,L) in Adult Human Primary Cardiomyocytes

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INa,L, a depolarizing current that persists throughout the action potential (AP) plateau, contributes to the AP duration and maintains intracellular Na^+ homeostasis. Increase or inhibition of INa,L is often associated with arrhythmogenicity or mitigation of pro-arrhythmia risk, respectively. Since drugs that block the hERG channel and also inhibit INa,L are not associated with pro-arrhythmia in humans, identifying the human effect of compounds on INa,L during preclinical development can aid in the determination of pro-arrhythmia risk for new drugs. In order to better understand properties of this current, we performed whole-cell voltage-clamp recording of INa,L using adult human primary cardiomyocytes isolated from ethically consented donor hearts. The current was examined by applying a pulse protocol every 5 seconds and measured using a ramp step. The data demonstrate that INa,L, under basal conditions, was activated at a membrane potential of -40mV and reached an average maximal current amplitude of -250pA around -10mV on the current-voltage relationship curve. Moreover, the basal INa,L was increased when cardiomyocytes were superfused with 0.06μM of the sea anemone toxin ATX-II, a well-known enhancer of INa,L (n=3). The stimulatory effect of ATX-II was reversible. Furthermore, we studied the effects of Ranolazine, an agent inhibiting INa,L, and found that 60μM Ranolazine inhibits basal INa,L (n=3). Moreover, in the continuing presence of ATX-II, Ranolazine elicited a pronounced reduction of INa,L (n=6). Thus, adult human primary cardiomyocytes express functional INa,L. Finally, AP and contractility experiments are currently underway to assess the actions of Ranolazine to suppress ATX-II- or hERG blocker-induced pro-arrhythmia. After a successful validation, human cardiomyocytes could potentially provide a useful strategy for the early assessment of the ability of new multichannel blocking drugs with INa,L affinity to prevent the occurrence of pro-arrhythmia.

PS 1702 Full-Time Control of Environmental Temperatures Reduces Edge Effect in Plate-Based Cell Assays

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A variety of approaches have been taken to avoid the variable results from edge wells in 96-well plates. The simplest approach is to avoid using them altogether, which eliminates 37% of the plate from the assay. This is tremendously wasteful of materials and time. Others have reported that incubation for extended periods of time at room temp during cell settling helps, although this is stressful for cells. We previously reported that plating cells at constant 37°C also reduces edge effect. Here we extend those studies to test the ability of constant thermal control of plating conditions to reduce variability in toxicity assays using human A549 lung and bone marrow Mesenchymal Stromal/Stem Cells (MSC). Our null hypothesis was that we would see no difference between the standard deviations in results from groups of wells including and excluding edge wells when comparing plates kept in uncontrolled room temp or controlled temp conditions. We used the Xvivo System to provide fully controllable gas and temperature levels for both cell incubation and handling. We performed 24-hour MSC toxicity assays with ethanol. Using the HoloMonitor M4 microscope, we showed that cell settling in edge wells was non-random when cells were plated at room temp and transferred to the incubator. MTT-based metabolic and crystal violet cell density assays showed that the cells were affected in a dose-dependent manner by ethanol. Data was normalized to its own control group and compared using a two-way ANOVA followed by Sidak's multiple comparisons test. Statistic analysis showed there was no significant difference between the group with and without the edge wells when plated at 37°C, while there was a significant difference in groups with and without edge wells when plating at RT. This suggests that variability in plate-based cell assays from use of edge wells can be reduced by plating the cells in controlled thermal conditions. Thermal imaging supports a model of heat transfer in which thermal currents form in the edge wells as cells settle

and the plate warms in the incubator, forcing cells toward the edges of the plate. We concluded that plating cells in constant 37°C may make it possible to use the whole plate, saving materials and time.

1703 Peripheral Neuropathy following a Single Exposure to a CXCR3-Depleting Antibody in Non-Obese Diabetic Mice

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The CXCR3 axis plays a role in autoimmune disease and CXCR3+ cell depletion shows promise as a treatment in autoimmune diseases such as vitiligo. During a study to assess the effects of CXCR3 depletion on diabetic NOD mice histopathology revealed that significant levels of inflammatory infiltrate into the sciatic nerve were noted in animals treated with a single dose of a commercially available hamster depleting antibody targeting murine CXCR3. Follow up work was conducted to further examine this finding. NOD and CD1 mice were treated with a single dose of 1, 5, or 40 mg/kg of the CXCR3 depleting antibody. Mice were sacrificed at 15, 50 or 100 days post dose and the sciatic and trigeminal nerves were microscopically examined for signs of inflammatory infiltrate and neuropathy. Immunophenotyping confirmed depletion of CXCR3 bearing cells. Nerve lesions were seen in NOD mice in all dose groups beginning at the 50 day post dose time point with no evidence of reversibility by the 100 day post dose time point. Lesions were seen in both the sciatic and trigeminal nerves, showing both inflammatory infiltrate as well as evidence of neuronal damage. Levels of NFLh (neurofilament heavy chain), a marker of structural nerve damage, were significantly higher in animals with nerve lesions. No lesions were seen in the CD1 mice at any dose or time point. A single dose of a CXCR3 depleting antibody was sufficient to induce a delayed peripheral neuropathy in NOD, but not CD1, mice that showed no signs of reversibility up to 100 days after treatment. A study in non-human primates was also conducted, and there was also evidence of inflammatory infiltrate into sciatic nerve in that study.

1704 The OrganoTEER: A Sensitive TEER Measurement Platform for High-Throughput Screening of Organs-on-Chips

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The study of epithelial barrier function is essential for fundamental understanding of disease processes, development of new therapeutic treatments, and investigation of compound safety. The gold standard barrier integrity measurement in epithelial models is the Trans Epithelial Electrical Resistance (TEER). The electrical properties of an epithelial layer are in a direct correlation to aspects such tight junctions, confluency, layer thickness and many morphological parameters. To address the lack of no commercially available solution for TEER on Organ-on-a-Chips, we developed a measurement apparatus to investigate epithelium cultured in a commercially available organ-on-a-chip platform. Up to 96 tubules cultured in a single OrganoPlate® have been studied by monitoring the diffusion of fluorescent probes (e.g. FITC-dextran) out of such tubes over time and calculating the apparent permeability (P_{app}). Here we investigate the relationship between TEER values measured using the OrganoTEER and P_{app} values determined using fluorescence based barrier integrity assays. We demonstrate the dynamic range for our TEER measurement method on epithelial tubes (CACO-2) including the response to toxicant exposure in comparison with Papp. The developed system makes use of an electrode interface compatible with the OrganoPlate 3-lane. Pairs are immersed in the wells connected to the inlet and outlet wells of each chip on the OrganoPlate. TEER values can be determined for up to 80 perfused tubules within 60 seconds. The instrument can be placed within an incubator to enable automated long term monitoring of TEER over the entire duration of an epithelial/endothelial study. Collagen I at 4mg/ml was layered in the gel compartment using Phaseguide technology. Caco2 tubules were formed against collagen I and under rocker-based perfusion flow. After 5 days of culture, we exposed to Staurosporine at varying concentrations (10uM, 2.5uM, 625uM, 156nM, 39nM, 9.7nM and 0nM). To investigate the relation between TEER and barrier integrity, we measured TEER on all tubules. We subsequently perfused them with a fluorescently labelled 10kDa Dextran dye and recorded its diffusion into the ECM channel using live fluorescent imaging. The results are expressed as TEER (fold change from before exposure) and P_{app} ($cm.s^{-1}$) respectively. As a result of staurosporine toxicant effect the P_{app} starts to increase at 20h exposures to 625nM staurosporine and higher (2.15e-5 +-0.79e-5 $cm.s^{-1}$ at 62nM), while TEER values was already decreasing relative to control at much lower dose and exposure times (relative change of 0.78+-0.09 at 156nM and 1 hour of exposure time, 0.38+-0.06 at 9.7nM and 20 h of exposure time). The increased sensitivity of this system is combined with the possibility to program label-free measurements throughout incubation without interruption of the experiment. We demonstrated the implementation of TEER measurements on an Organ-on-a-Chip platform and compared it to live imaging based permeability measurements. Based on these results, we propose this system for the study of healthy and damaged models of 3D barrier tissues in a non-invasive way to provides a valuable tool for drug toxicity and transport studies in Organ-on-a-Chip models.

1705 Benchmarking *In Vivo* Toxicokinetic Data against Complex *In Vitro* Models to Predict Drug Induced Liver Injury

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Drug induced liver injury (DILI), is a leading cause for attrition during drug development and one of the main reasons drugs are withdrawn from the market. DILI is the most common cause of death from acute liver failure and accounts for approximately 13% of cases of acute liver failure in the United States. There are currently no reliable methods to screen compounds for DILI preclinically, and the specifications used during high-throughput screening are often limited in scope. The need and importance to accelerate drug discovery, including the detection of DILI, has increased, as has the need to bring together expertise and resources from multiple disciplines and organizations. The Accelerating Therapeutics for Opportunities in Medicine (ATOM) consortium is a government, industry, and academic partnership with the goal of rapidly accelerating drug discovery by integrating high-performance computing, deep learning, and human-relevant complex *in vitro* models. ATOM is leveraging emerging phenotypic *in vitro* screening assays to generate data and creating computational algorithms to correlate *in vivo* biomarkers to *in vitro* data. In order to validate the computational models and the *in vitro* assays, data from GSK historical preclinical rat toxicity studies are used as a ground truth benchmark. This data includes histopathology, pharmacokinetic, and clinical chemistry data from 200 compounds that have been terminated. The conclusions drawn from the algorithms created and correlations found between the *in vivo* and *in vitro* datasets will allow us to establish guidelines and strategy for future toxicity screening studies in a systematic and reproducible way.

1706 An *In Vitro* Evaluation of Cytotoxicity and Mitochondrial Toxicity of Anti-Retroviral (ARV) Drugs in HepG2, SNU-475, and K562 Cells

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Mitochondrial toxicity (MT) is responsible for curtailed use and post-market withdrawal of many drugs; however, there is no one assay available that reliably predicts MT in early drug screening activities. We previously developed a panel of *in vitro* assays that encompass three major adverse events resulting from MT: (1) energy metabolism disruption, (2) increased oxidative stress, (3) altered apoptosis. This panel successfully characterized MT of nucleoside reverse transcriptase inhibitors (NRTI) in ten cell lines derived from liver, kidney and heart tissues. Here, we evaluated the *in vitro* cytotoxicity and MT of ARV drugs elvitegravir (EVG) and raltegravir (RTG) in three cell lines: HepG2 (liver), SNU-475 (liver) and K562 (bone marrow) with zalcitabine (ZTB) as a positive control. The *in vitro* cytotoxicity at six concentrations of EVG (0.1- 200μM), RTG (0.1-50μM) and ZTB (0.1 - 100μM) was evaluated for 72 hr by MTT assay. The EVG at concentrations higher than 10 μM resulted in ≥ 50% loss of cell viability in all three cell lines while no impact on cell viability was observed following 72-hr treatment with RTG. A ~20% loss of cell viability in all three cell lines was observed at 100μM ZTB. Based on cell viability data, three concentrations of EVG (10, 5 and1μM), RTG (50, 10 and1μM) and ZTB (100, 50 and 10μM) were selected for evaluating their MT potential. The cells were cultured with each compound for two weeks with cells harvested on Days 7 and 14 post incubation and tested in the *in vitro* assay panel. The 14-day treatment of three concentrations of RTG did not adversely impact the mitochondrial OXPHOS 1, 3-5 protein, caspase 3 and ROS/RNS levels in all three cell lines. The 14-day EVG treatment did not adversely impact the OXPHOS levels in HepG2 cells; however, a 20-100% decrease in OXPHOS1, 3-5 levels were observed in SNU-475 and K562 cells at 10 and 5μM. The 14-day ZTB treatment

resulted in a 30-100% decrease of OXPHOS 1, 3 and 4 levels in all three cell lines which confirms our previous results. *Work supported by NIAID Contract HHSN272201400006I.*

1707 Aircraft Cabin Fume Events: Is There a Toxicological Explanation?

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Cabin air is derived in most aircraft from the compression stage of the jet engine. This bleed air is conditioned and filtered, with an exchange of 10-15 times per hour with outside air and 20-30 times per hour including outside and filtered recirculated air. On rare occasions, it is possible for the conditioned air to be contaminated by trace amounts of engine or hydraulic oil because of bearing failure or over-filling of the oil reservoir, giving rise to a "fume event" (visible smoke, haze, and/or odors) estimated to occur on 0.05% of flights overall (1 in 2,000). This Workshop will review firstly those studies that have analyzed the air of civilian and military aircraft and detected not only compounds traceable to various aviation fluids (jet fuel, lubricants, hydraulic fluid, and coolants) but also pyrethroids and ozone. Secondly, it will assess the toxicity of these identified chemicals to humans. Thirdly, a large investigation of 2,674 cabin crew conducted by the German Federal Institute for Risk Assessment (BfR) will be reported, and fourthly, a critical assessment of whether analytical investigations can assist in the diagnosis of cabin fume events will be presented. Some studies have detected tri-cresyl phosphate, an organophosphate used as a high-pressure lubricant in engine oil. However, the four meta/para isomers of tri-cresyl phosphate, which account for 99.8% of all isomers, are not neurotoxic. The six ortho-isomers account for the remainder. There is particular concern that tri-ortho-cresyl phosphate, which is bioactivated by CYP 1A2 and 3A4 to 2-(ortho-cresyl)-4H-1,2,3-benzodioxaphosphoran-2-one (CBDP), may be responsible for neurological sequelae, as this metabolite can bind both to human acetylcholinesterase (producing acute organophosphorus poisoning) and to Neuropathic Target Esterase (NTE), thereby initiating organophosphate-induced delayed neuropathy. Pyrethroid spraying on some flights to help ameliorate airborne diseases leads to clinically significant pyrethroid concentrations, which could result in nasal irritation, throat irritation, sneezing, cough, dizziness, headache, and nausea. High ozone concentrations, which are well documented, particularly during intercontinental polar flights in late winter/early spring, could produce eye, ENT, and respiratory features, such as dry mouth or lips, dry and itchy eyes, and nasal stuffiness. In the BfR study, the majority (76%) of the crew complained of odors similar to the smell of oil, "used socks," or burned material. The symptoms most frequently experienced were headache, dizziness, and nausea (>20% each). A smaller percentage (< 20%) of the patients reported symptoms such as "prickly sensations" (paresthesiae) and numbness. An adduct of CBDP with butyrylcholinesterase was first reported to be present in the serum of a group of asymptomatic aircraft passengers in 2011. More recently, adducts of the cresyl-benzodioxa-phosphorin-oxide-derived phosphyl group with tyrosine residues have been detected as well as histidine- and lysine-adducts with ortho-cresyl. Such peptides have been used as biomarkers in diverse smaller studies and case reports. However, these analytical methods are extremely sensitive, and a correlation between these biomarkers and development of signs and symptoms has not yet been firmly established.

1708 Is There a Toxicological Explanation for Features Following Cabin Fume Events?

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It is possible for cabin air to be contaminated by various aviation fluids (jet fuel, lubricants, hydraulic fluid, and coolants), pyrethroids, and ozone. Tri-cresyl phosphate is present as a high-pressure lubricant in engine oil. However, the four meta/para isomers of tri-cresyl phosphate, which account for 99.8% of all isomers, are not neurotoxic. The six ortho-isomers account for the remainder: three are mono-ortho isomers, two are di-ortho isomers, and one is the tri-ortho isomer (tri-ortho-cresyl phosphate), which is bioactivated by CYP 1A2 and 3A4 to 2-(ortho-cresyl)-4H-1,2,3-benzodioxaphosphoran-2-one (CBDP). This metabolite can bind to human acetylcholinesterase and Neuropathic Target Esterase (NTE). As a result, exposure to substantial amounts of tri-ortho-cresyl phosphate (by ingestion not inhalation) has led to features of acute organophosphorus poisoning due to inhibition of acetylcholinesterase and to organophosphate-induced delayed neuropathy from > 70% inhibition of NTE. Ozone enters the cabin from outside the aircraft, particularly during intercontinental polar routes, and during late winter/early spring. When first installed, ozone catalytic converters decompose 90%-98% of the ozone present, but converters lose their efficiency because of other cabin contaminants ("surface poisoning"). High ozone concentrations could produce eye, ENT, and respiratory features, such as dry mouth or lips, dry and itchy eyes, and nasal stuffiness. Pyrethroid spraying (pre-flight, immediately before takeoff, and at the top of descent) on some flights to help ameliorate airborne diseases leads to clinically significant pyrethroid concentrations, which could result in nasal irritation, throat irritation, sneezing, cough, dizziness, headache, and nausea.

1709 Of Which Symptoms Do Cabin Crew Complain after a Cabin Fume Event?

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German physicians are required by law (Chemikaliengeset § 16e) to report cases of poisoning to the Federal Institute for Risk Assessment (BfR). This obligation also applies to individuals involved in a cabin fume event. Between 2009 and 2018, 1,027 cabin fume events involving 2,674 individuals were reported to the Federal Institute for Risk Assessment. All cases concerned air crew members. Women (59%) were involved more frequently than men (37%), and more flight attendants (64%) reported exposures than pilots (20%). The majority (76%) of the individuals complained of odors, mainly similar to the smell of oil, "used socks," or burned material. The symptoms most frequently experienced were headache, dizziness, and nausea (>20% each). A smaller percentage (< 20%) of the patients reported "prickly sensations" (paresthesiae) and numbness. 20% of individuals did not report any symptoms (WHO/IPCS/EC/EAPCCT Poisoning Severity Score [PSS] of 0), 66% had a PSS of 1 (minor symptoms), and 5% had a PSS of 2 (moderate symptoms). No severe symptoms (PSS 3) were reported. Patients with PSS 2 complained most commonly of severe or prolonged headaches, cognitive impairment, syncope, hypertension, or repeated vomiting. Laboratory results such as cholinesterase activity, oxygen saturation, carboxyhaemoglobin, and methaemoglobin concentrations did not show abnormal values indicating poisoning. In conclusion, symptoms reported were in general nonspecific and variable between patients. Due to this variability and lack of biomonitoring data, it was not possible to match symptoms with potential toxic agents.

1710 Can Analytical Investigations Assist in the Diagnosis of Cabin Fume Events?

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Tri-ortho-cresyl phosphate has been suggested as a component that might be present following cabin fume events. Although possible toxic mechanisms might be mediated by binding to acetylcholinesterase or neuropathy target esterase, only high-level inhibition of these enzymes is associated with clinical effects. A further possible target of cholinesterase inhibitors, the plasma—or butyrylcholinesterase (BChE)—shows a huge intra- and inter-individual range activity, preventing a clear assessment at low-level inhibition. Hence, more sensitive methods are needed for assessment of low-level exposure. The detection of adducts formed with cresyl-benzodioxa-phosphorin-oxide—an activated biotransformation product of tri-ortho-cresyl phosphate—with either BChE or serum albumin can be used for monitoring. BChE is present in plasma in concentrations as low as 3-4 mg/L, thus requiring its extraction from the complex plasma matrix. BChE isolation may be performed by affinity chromatography using procainamide or by immunomagnetic separation. The latter technology makes use of paramagnetic particles (beads) labeled with monoclonal antibodies against BChE that are directly added to plasma. With subsequent incubation, BChE is bound to the beads. Following enzymatic proteolysis using chymotrypsin or pepsin, small peptides are generated. If *in vivo* exposure to tri-ortho-cresyl phosphate has occurred, a characteristic phosphyl moiety is attached covalently to the serine residue of the esteratic center of BChE. This modified peptide is separated by liquid chromatography and detected by tandem mass spectrometry (MS/MS) with high sensitivity and selectivity. In addition to BChE, adducts to serum albumin may be monitored. Adducts of the cresyl-benzodioxa-phosphorin-oxide-derived phosphyl group with tyrosine residues can be detected as well as histidine- and lysine-adducts with ortho-cresyl following enzymatic degradation catalyzed by either pepsin or trypsin. Such peptides have been used as biomarkers to detect tri-ortho-cresyl phosphate exposure in pilots and flight attendants in diverse smaller studies and case reports. However, these analytical methods are extremely sensitive, and a correlation between these biomarkers and the development of signs and symptoms has not yet been established.

1711 Globally Harmonized Guidance for Dose-Response Analysis and Derivation of Health-Based Guidance Values for Chemicals in Food. Part I—What's New

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The World Health Organization (WHO) recently coordinated an international effort to update its guidance document on dose-response assessment for chemicals in foods, including additives, contaminants, natural toxicants, and residues of pesticides and veterinary drugs. This guidance also provides practical recommendations in deriving health-based guidance values, such as acceptable daily intake and acute reference dose, as the basis of establishing food safety regulations and safety standards in international food trade. Although the purpose of the guidance is related to the WHO food safety programs, the guidance reflects the latest expert thinking on dose-response assessment and benchmark dose (BMD) modeling, including some key areas of consensus among international experts, and so will be broadly applicable to areas beyond chemicals in food. Key motivators for the update were newly available modeling technology and the need for harmonization of the BMD approaches used by the US Environmental Protection Agency (US EPA) and the European Food Safety Authority (EFSA). This two-part Workshop includes presentations and demonstrations given by scientists who played essential roles in an international working group charged with drafting this harmonized guidance. Part I of the Workshop will focus on the basic elements and key updates in the new guidance, while Part II is designed as a practical demonstration session that builds on presentations in Part I. Part I will begin with an introduction that provides an overview of this guidance, the objectives of the working group, and a summary of notable updates. The second presentation will address the need for global harmonization with some food-related case studies and will present the new consensus decision tree scheme for dose-response analysis designed to aid in harmonizing approaches. The decision tree scheme, embracing a new tiered approach for the benchmark response for continuous endpoints, provides a practical guide to risk assessors and managers by illustrating how key decision points are affected by a variety of practical scenarios. The third presenter, who played a leading role in the incorporation of model averaging into the US EPA BMD software, will focus on the harmonization of BMD methodology by specifically outlining important considerations in the practice of BMD, explaining recommendations of modeling strategies for different types of data, and presenting challenges and solutions in harmonizing different methods. The session will conclude with an interactive panel discussion with all speakers, Chairs, and a moderator who collectively represent end users of this guidance from different sectors.

1712 Bring Together Multidisciplinary Expertise: Highlights of Updates in the New Dose-Response Analysis Guidance for Chemicals in Food

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For chemicals in food, health-based guidance values (HBGVs), such as acceptable or tolerable daily intakes and acute reference doses, represent a range of oral exposures that are expected to be without appreciable health risk. The WHO original guidance, "Principles and Methods for the Risk Assessment of Chemicals in Food" (i.e., Environmental Health Criteria [EHC] 240), had limited context related to benchmark dose modeling, a field that has since rapidly evolved into different perspectives and approaches. By convening toxicologists, epidemiologists, mathematical modelers, risk assessors, and regulators, a multidisciplinary approach was used to update the guidance to provide a practical and harmonized recommendation to perform dose-response assessment (DRA) and establish HBGVs. Notable updates to the contents include dose-response modeling methodology (e.g., software, statistical issues, model evaluation criteria), a tiered approach to determining a benchmark response (BMR); a decision tree diagram for DRA leading to the establishment of HBGV or margin of exposure, new sections on dealing with epidemiological and *in vitro* data, incorporating new methods to derive chemical-specific adjustment factors, and revised terminology to improve consistency, clarity, and accuracy. The update also includes an appendix of examples based on dichotomous, continuous, and epidemiological data and additional information and references detailing model components, statistical fitting methods, and uncertainty. This overview will set the stage for the subsequent speakers to illustrate important technical applications of the new guidance, including choice of BMR and how model averaging and Bayesian approaches minimize issues related to model choice.

1713 Step by Step: Decision Tree for Dose-Response Analysis and Choice of the Benchmark Response

L. Haber. *TERA Center, University of Cincinnati, Cincinnati, OH.*

Key challenges in dose-response assessment have been disagreement on when it is appropriate and necessary to conduct dose-response modeling and differences in the specifics of the modeling guidance from different organizations, particularly the US EPA and European Food Safety Authority (EFSA). The importance of these differences and the need for harmonization are illustrated using case studies of two chemical food contaminants: 3-monochloropropane-1,2-diol, a contaminant in (3-MCPD) (impact of restricting models) and 5-hydroxymethylfurfural (HMF) (implications of considering visual fit, the Akaike information criterion [AIC], and scaled residuals in choosing models). An important aspect of the new guidance is a decision tree designed to aid risk assessors and managers and lead to a harmonized approach. The decision tree, agreed upon with full consensus at a meeting of international experts convened by WHO in March 2019, lays out the key steps in the dose-response assessment. Steps for conducting benchmark dose modeling and non-modeling approaches are addressed. The decision tree introduces a tiered approach for choosing the BMR for continuous endpoints, including (1) using a biologically meaningful adverse effect size; (2) expert judgment on the quantitative definition of adverse; and (3) using dose-response data to calculate a margin of exposure. Examples of application of the tiering will be provided.

1714 Bring Harmonization into Dose-Response and Benchmark Dose Modeling Guidance

M. Wheeler. *CDC, Cincinnati, OH.* Sponsor: Y. Zang

To establish a point of departure, some form of dose-response analysis (DRA) must be employed. Benchmark dose (BMD) modeling is the preferred approach over the NOAEL/LOAEL approach for a number of reasons, as identified by the WHO risk assessment guidance EHC 240. However, there are myriad choices available to the modeler, which range from the form of the dose-response curve to the type of analysis (e.g., frequentist versus Bayesian versus model average), and the question becomes, How does one make reproducible, scientifically valid decisions with all of these options? To bring harmonization and transparency into BMD modeling, the new guidance addresses these discrepancies by emphasizing the use of multi-model inference using model averaging, with a reliance on Bayesian models to implement "soft-constraints" on model parameters. The speaker will focus on how the newly developed guidance leverages new methodologies (e.g., model averaging and Bayesian dose-response modeling) while producing scientifically sound and robust results. Emphasis is made on the selection of different model suite and Bayesian priors for proper analyses of the BMD. This talk is geared toward toxicologists and BMD software users. It focuses on the practical choices when performing a dose-response analysis and is not intended to be technical in nature.

1715 Impacts on Human Health of Sunscreen Active Ingredients Used to Filter UV Light

M. Hughes. *US EPA, Research Triangle Park, NC.*

There are several known adverse health effects in the skin following overexposure to ultraviolet (UV) light from the sun. Acute effects from UV light overexposure include erythema, immunosuppression, and burns; chronic effects include photoaging and cancer. Occurrence of skin cancer from overexposure to sunlight is on the rise, alarming many health agencies. Total avoidance of sunlight and the wearing of protective clothing are the best ways to evade the harmful effects of sunlight. However, these two practices are not always the most practical way to avoid sunlight. In addition, excessive avoidance of sunlight may result in vitamin D deficiency, which has its own adverse effects, such as risk for cardiovascular disease and cancer. A more convenient means of protection from UV light from the sun is the application of sunscreen onto exposed skin. Sunscreens are basically inorganic (physical) and organic (chemical) filters of UV light. The main types of inorganic sunscreens or UV filters are zinc oxide and titanium dioxide, while several chemical classes are used as ingredients of organic sunscreens. Examples include the benzophenones, cinnamates, and others. These organic UV filters are not in sunscreens alone but also are found in other personal care products such as cosmetics and hair sprays, and clothing. Human exposure to the UV filters is primarily topical but can occur via the lungs after using spray applicators or by the gastrointestinal tract after use of lip balms containing these chemicals. Concerns have been raised about the potential adverse health and environmental effects of the UV filters. For example, the benzophenone derivative, 2-hydroxy-4-methoxybenzophenone (i.e., oxybenzone, benzophenone-3), is

one of the most predominant organic chemical-based ingredients of sunscreens currently used in the United States. Oxybenzone is detected in raw and treated wastewater and in coastal marine waters of Hawaii at low part per trillion concentration. Oxybenzone has been reported to be potentially photoallergenic, have endocrine-like effects in laboratory animals, and have a negative impact on marine organisms such as coral. The latter effect and its detection in Hawaiian waters has led the legislature of Hawaii to ban the use of oxybenzone-containing sunscreens by 2021. This session will focus on the potential human health effects from exposure to sunscreen active ingredients such as the benzophenones and cinnamanates. The first presenter will provide an overview of the dermatotoxicology of sunscreen active ingredients, with regard to past and future regulatory aspects of sunscreen active ingredients, as well as their safety and efficacy. The second presenter will summarize the results of the National Toxicology Program's efforts to characterize potential 2-hydroxy-4-methoxybenzophenone-related endocrine and developmental toxicity and carcinogenicity. Study designs utilized include *in vitro* and short-term *in vivo* (Endocrine Disruptor Screening Panel), rat multigenerational (including teratological), and two-year rodent carcinogenicity. The third speaker will present evidence on the effect of UV filters on the migration of human breast cancer cells. This effect has implications on breast cancer development and metastasis to other tissues in humans exposed to UV filters. *This abstract does not represent US EPA policy.*

W 1716 Sunscreen Dermatotoxicology: Past and Future

H. I. Maibach. *University of California San Francisco, San Francisco, CA.*

UV light exposure from the sun to human skin has a beneficial effect, the biosynthesis of Vitamin D, which is vital for normal human function. In contrast, overexposure to UV light has adverse effects, such as sunburn, early aging of skin, and skin cancer. Almost 90% of all skin cancers may be attributed to UV light exposure. Over the past several decades, the rate of melanoma, a type of skin cancer caused by UV light, has risen fivefold in Caucasian populations. The use of sunscreens can limit overexposure to UV light. They contain chemical ingredients that reflect and absorb UV light. The first commercial sunscreen was introduced in the 1920s, after it was found that UV light can damage skin. Fifty years later, the US Food and Drug Administration (US FDA) proposed a rulemaking to institute provisions for the safety, efficacy, and labeling of sunscreens. US FDA considers sunscreen ingredients as over-the-counter drugs. A final rule in 1999 established conditions where sunscreens would be generally recognized as safe and effective. In 2019, US FDA proposed to update the sunscreen regulations. This was due to the use of sunscreen with a greater number of UV light-blocking ingredients and at higher concentrations, the increased encouragement for sunscreen use, and an increased number and type of products used daily that contain sunscreens. Also, the safety of several ingredients and the extent of their systemic absorption after topical application has been questioned. Evidence suggests that the ingredient oxybenzone has endocrine activity. For systemic absorption, in the 1960s, when analytic chemistry sensitivity permitted, it became clear that the leading sunscreen of that time, para-amino benzoic acid, penetrated human skin *in vivo* in amounts equal or exceeding that observed with drugs. A recent clinical trial showed that four currently used sunscreen ingredients, including oxybenzone, had systemic concentrations greater than the US FDA guidance level of 0.5 ng/mL. These concentrations were reached after four topical applications of the ingredients on day 1 of the study. As sunscreens are used almost lifelong, we posit that their safety deserves careful evaluation. The presentation summarizes current data for US-utilized sunscreens and elaborates on data gaps, some readily obtainable and others important but not yet in progress.

W 1717 *In Vitro* and *In Vivo* Studies Assessing the Potential for 2-Hydroxy-4-methoxybenzophenone (HMB) to Affect Endocrine Activity, Reproductive Performance and Fetal Development, Endocrine-Sensitive Tissue Growth, and Tumor Incidence

B. S. McIntyre, E. Mutlu, S. Waidyanatha, H. Cunny, and M. Stout. *NIEHS/NTP, Research Triangle Park, NC.*

The National Institute of Environmental Health Sciences National Toxicology Program (NIEHS/NTP) has been studying the potential for UV sunscreens to induce adverse responses in model systems that are considered informative and predictive of potential human risk. Possible estrogenic and androgen/antiandrogenic effects of HMB (oxybenzone, benzophenone-3) were apically evaluated utilizing the Endocrine Disruptor Screening Panel Study designs. The goal of these *in vitro* and *in vivo* protocols is to determine if the test article affects estrogen or androgen receptor (ER and AR) binding, activation, synthesis, or metabolism. Potential *in vivo* estrogenic activity was assessed in the uterotrophic assay, and androgenic and antiandrogenic activity are evaluated in the Hershberger assay. Using ERs isolated from rat uteri, HMB was classified as "non-interacting." HMB displayed weak ER agonist activity with a LogPC10 of 5.5 Log[M] in the HeLa-9903 system. HMB did not induce a uterotrophic response and was classified as "equivocal" in the aromatase inhibition assay. Using ARs isolated from rat prostate, HMB was classified as an "equivocal" binder. HMB displayed antagonism of AR-mediated transactivation at ~10-4 M in the MDA-kb2 cell assay and was negative in the rat Hershberger assay. Potential HMB-mediated reproductive and developmental toxicity was assessed using the NTP Modified One-Generation study design. In this design, F1 HMB exposure is continual, beginning at the time of implantation. Endpoints examined included those that are sensitive to AR or ER modulation (e.g., anogenital distance, vaginal opening) as well as potential effects on F0/F1 reproductive performance, and developmental outcomes in the F2 generation. HMB exposure via dietary administration up to 30,000 ppm had no effect on F0 or F1 reproductive performance or anogenital distance, areolae/nipple retention (in either the F1 or F2 offspring), vaginal opening, or balanopreputial separation. HMB exposure was associated with increases in adult liver and kidney weight, and concomitant gross and histological lesions, in both sexes. There were no HMB-related effects on fetal weight or the incidence of adverse external, visceral, or skeletal findings. HMB was assessed in two-year rat and mouse carcinogenicity studies via dietary administration up 10,000 ppm. There were no apparent HMB-related increases in the tumor incidence of hormone-dependent tissues.

W 1718 Effects of Chemical Ultraviolet Filters on Proliferation, Migration, and Invasion of Human Breast Cancer Cells *In Vitro* at Concentrations Measured in Human Breast Tissue

P. D. Darbre. *University of Reading, Reading, United Kingdom.* Sponsor: M. Hughes

The human population is exposed to UV filters not only from their use in sunscreen products but also from their widespread addition to other personal care products and textiles to increase product stability. Our recent research has aimed to measure concentrations of some commonly used UV filters in human breast tissue, and then to determine whether those concentrations are sufficient to enable measurable effects in human breast cancer cells *in vitro*. HPLC-MS/MS was used to measure concentrations of benzophenone-3 (BP-3), octylmethoxycinnamate (OMC), and 4-methylbenzilidenecamphor (4-MBC) in human breast tissue taken from three serial locations across the breast from 40 women undergoing mastectomy for primary breast cancer. One or more of these UV filters were quantifiable in 101 of 120 (84%) of the tissue samples and at least one breast region for 38 of the 40 women. BP-3, OMC, and 4-MBC were detected in 69%, 74%, and 13% of samples, respectively. Using cell culture models, each of these three UV filters could increase proliferation of oestrogen-responsive human breast cancer cells at 10-7 M concentrations, which equate to levels measurable in some of the breast tissue samples. Following long-term exposure, these UV filters also increased cell migration and invasion at 10-7 M concentrations but did so in oestrogen-unresponsive as well as oestrogen-responsive human breast cancer cells. Given that many of these same breast tissue samples contained not one but multiple UV filters and together with several of the esters of p-hydroxybenzoic acid (parabens), the full potential for development of hallmarks of cancer in breast cells *in vivo* requires consideration to mixtures of these pollutant chemicals.

R 1719 Mechanistic Read-Across of Chemical Toxicants Based on Big Data

H. Zhu. *Rutgers, The State University of New Jersey, Camden, NJ.*

In 2016, the Frank R. Lautenberg Chemical Safety for the 21st Century Act became the first US legislation to advance chemical safety evaluations by utilizing novel testing approaches that reduce the testing of vertebrate animals. Central to this mission is the advancement of computational toxicology and artificial intelligence approaches to implementing innovative testing methods. In the current "big data" era, the volume (amount of data), velocity (growth of data), and variety (diversity of sources) are critical considerations when characterizing the currently available chemical, *in vitro*, and *in vivo* data for toxicity modeling purposes. Furthermore, as suggested by various scientists, the variability (internal consistency or lack thereof) of publicly available data pools, such as PubChem, also presents significant computational challenges. The development of novel artificial intelligence approaches based on massive public toxicity data is urgently needed to generate new predictive models for chemical toxicity evaluations and establish scientific confidence in

the developed models as alternatives for evaluating untested compounds. In this procedure, traditional approaches (e.g., QSAR) purely based on chemical structures have been replaced by newly designed data-driven and mechanism-driven modeling. The resulting models realize the concept of adverse outcome pathway (AOP), which can not only directly evaluate toxicity potentials of new compounds but also illustrate relevant toxicity mechanisms. The recent advancements of computational toxicology in the big data era are paving the road to future toxicity testing and will have significant impacts on public health.

1720 Individual-, Community-, and Population-Level Environmental-Associated Attributes of Disparate Health Outcomes in Vulnerable Populations

D. Hood. *Ohio State University, Columbus, OH.*

While life expectancy in the US has increased significantly over the past five decades, key indicators of chronic disease health outcomes continue to vary markedly by race, ethnicity, sex and/or gender, geographic location, and socioeconomic status (SES). Disparate health outcomes are well documented and account for a substantial proportion of preventable death and disability. In population groups defined by race/ethnicity, sex and/or gender, geography, and/or SES, disparities persist, and in some settings, they are widening. This Informational Session will present an innovation in the science of health disparities known as the Public Health Exposome (PHE) framework and its applications. This framework was devised as a means to identify important associations between chemical and nonchemical stressor exposures and chronic disease outcomes in vulnerable populations using both data-driven and hypothesis-driven methods. Using the Public Health Exposome dataset 3.0, the session will demonstrate how to use data-driven computational and hypothesis-driven statistical analytics to discover new scientific insights into associations between the natural, built, and social environments, adverse personal health outcomes, and disparate population-level health disparities. The PHE framework offers a new approach for conducting cumulative risk assessments that can be used to assess the effects of multiple, interactive, and cumulative chemical and nonchemical stressor exposures on disparate health outcomes and on population-level health disparities. By curating large amounts of disparate, heterogeneous data, the PHE approach provides environmental public health scientists, epidemiologists, and chronic disease researchers with the tools to develop, target, and evaluate current and past public health programs and policies.

1721 The Epidemic of Chronic Kidney Disease of Unknown Etiology in Agricultural Communities

A. Harrill. *NIEHS/NTP, Research Triangle Park, NC.*

Over the last two decades, occurrence of severe renal disease has dramatically increased in socially vulnerable farming communities within Central America, Sri Lanka, and India—and most recently reported in the United States. In countries such as Nicaragua and Costa Rica, chronic kidney disease mortality is over fivefold the national rates and increasing, with an overall mortality >20,000 young and middle-aged agricultural workers in affected regions. The epidemic of chronic kidney disease of unknown origin (CKDu) is not associated with classic CKD risk factors such as diabetes and hypertension. Potentiating factors are beginning to be explored, including heat stress and dehydration resulting from strenuous work in hot climates, agrochemical and metal exposures in occupational settings, and through drinking water sources. There is some evidence indicating that repeated episodes of acute kidney injury occurring during the growing season may have a cumulative effect in precipitating chronic kidney disease. A key question is whether the cause of the disease is the same worldwide or whether different factors are leading to the same manifestation in various disease hot spots. For example, well water as a primary drinking source has been implicated as a risk factor for the disease in Asian countries, but there is not sufficient evidence linking drinking water exposures elsewhere. What is clear is a strong association with strenuous work in primarily agricultural occupations. Pathologic features of the disease indicate a possible toxic nephropathy, yet systematically collected exposure data are sparse but ongoing. In this session, the key features and underlying renal pathology will be reviewed along with early investigations toward assessing the evidence of an environmental toxic mediator. A panel discussion serves as a "call to action" to engage toxicologists to consider studies that will help to mitigate the serious human health catastrophe of CKDu in economically disadvantaged communities.

1722 Evolving Technologies for Determination of Biotherapeutic Specificity

T. MacLachlan. *Novartis Institute for BioMedical Research, Cambridge, MA.*

Monoclonal antibody therapeutics evolved from low molecular weight drugs with the promise of substantially higher specificity than low molecular weight drugs, and thus with much lower potential for toxicity. Since safety of monoclonal antibodies is directly linked to the specificity of their binding to therapeutic targets, developers of monoclonal antibody therapeutics have evaluated specificity of their products using the "tissue cross-reactivity" assay, based on the immunohistochemical method, where the drug candidate is panned across a large number of human tissues. Recently, several platforms for screening for potential cross-reactivity have become available, from protein and cellular arrays to flow cytometry-based methods, and their utility as possible replacements for the immunochemistry method has been considered. This session will address the growing options that sponsors have to evaluate specificity of monoclonal antibody pharmaceuticals in the preclinical safety package.

1723 "TCR 2.0": Reviewing Experience with Methods to Detect Off-Target Binding of Monoclonal Antibodies

J. Cavagnaro. *Access BIO LC, Boyce, VA.*

Monoclonal antibodies (mAbs) and derivatives thereof have become a mainstay pharmaceutical modality. A primary benefit of these proteins is their highly targeted nature, which can reduce the incidence of side effects in patients. It is critical, however, to ascertain the specificity of antibodies prior to human clinical trials, and several country- and worldwide guidances direct sponsors to evaluate this. For the last two decades, the primary technique for determining specificity has been the immunohistochemistry (IHC)-based tissue cross-reactivity assay (TCR), where the candidate antibody is panned across 32 tissues to look for unexpected staining. In the last few years, however, other array-based platforms have emerged that allow for screening the majority of the human membrane proteome, indicating a viable alternative and/or addition to the IHC methods. Additionally, a "Q&A" to the ICH S9 guidance in 2018 indicated that TCR assays are not required in most situations for oncology biotherapeutics. The preclinical committee of the Biotechnology Innovation Organization (BIO), "Biosafe," has conducted a survey of 26 BIO member companies to understand current sponsor experience with the IHC and array techniques for determining antibody specificity. In the last 10 years, more than 650 IHC TCR assays have been conducted largely on full-length mAbs with varying impacts on programs. Protein/cell arrays have been utilized by a third of the companies surveyed and are gaining familiarity and comfort with the platform—initial experience with recent versions of these arrays has been largely positive and are integrating new modalities such as scFvs from CAR-T therapies. ICH S6(R1) guidance offers sponsors the option to use alternatives to IHC to determine antibody specificity—while most sponsors are not prepared to eliminate the IHC method, growing experience with these alternatives may allow them to confidently choose one with or without IHC in the future. Details on sponsor responses and experience will be shared in this session.

1724 Immunohistochemistry-Based Tissue Cross-Reactivity Studies: History and Current Perspective

B. Buetow. *Pfizer Inc., San Diego, CA.* Sponsor: T. MacLachlan

Immunohistochemistry (IHC) has traditionally been used as the primary method to assess tissue cross-reactivity (TCR) for monoclonal antibody and antibody-based therapeutic candidates. More recently, TCR studies have been used to determine the potential cross-reactivity of more novel therapies such as T cell-engaging bispecifics and CAR-T cells, which can have potent and potentially long-lasting effects. TCR studies have been recommended since the early 1980s by various regulatory guidance documents, which have been modified over time to the most recent "Points to Consider" (US FDA, 1997) and ICH S6(R1) (2011) documents. TCR studies incubate the candidate antibody/molecule with a wide range of tissues and detect binding of the candidate antibody using a variety of IHC techniques. In this regard, TCR studies using IHC have the unique ability to test for cross-reactivity across many tissues from both humans and animals. An evaluation can be made about which cell type is involved, as well as whether the binding appears to be on the cell membrane versus cytoplasm. While TCR studies sometimes provide key information about potentially cross-reactive targets, they also are prone to false positive results (as assessed by the presence of findings in "positive" tissues in *in vivo* toxicity studies), which can have a negative impact on drug

development. This is in part because the candidate antibodies/molecules are selected for their ability to have a desired therapeutic effect *in vivo*, and not because they are good IHC reagents. Several case examples will be presented to highlight the variable results obtained with TCR studies.

1725 Protein and Cell-Based Arrays to Assess Specificity of Biotherapeutics

A. Vicart. *Novartis Institute for BioMedical Research, Basel, Switzerland.* Sponsor: T. MacLachlan

Off-target binding of biotherapeutics to either cell surface or secreted proteins can lead to attrition in drug development. With new modalities such as antibody drug conjugates (ADCs), bispecific antibodies targeting T cells and CAR-T therapies, a lack of specificity could lead to severe side effects in patients. In the last few years, protein and cell array-based platforms have emerged that allow for screening against an extensive range of human proteins. We have and are continuing to test various platforms, including non-contact inkjet printed protein arrays and cellular arrays in slide, plate, and flow cytometry formats. While some platforms are still under evaluation, we have implemented a revised strategy to profile the specificity of biotherapeutics, including (1) profiling cross-reactivity potential on a cellular array prior to formal toxicity studies, (2) immunohistochemistry on a panel of tissues using an IHC-optimized tool antibody against the intended target, and (3) including screening results in the IND package together with the formal IHC-based TCR for non-oncology indications or as a substitute to TCR for oncology or when an immunohistochemistry method was not able to be developed. Initial experience with this strategy with more than 90 molecules profiled has been largely positive. Undisclosed on-targets were identified in more than 90% of screens and 2/3 of identified off-targets were confirmed by alternative binding and/or functional assays. Several examples will be shared, including its use for IND submissions and regulatory acceptance of these approaches, as well as how it helped selecting the best candidate or terminate nonselective lead biotherapeutics with financial and animal use reduction impacts.

1726 Genome Editing in Drug Discovery: Using the Cas9 Protein to Explore Target-Mediated Toxicity

J. Willy. *Vertex Pharmaceuticals, San Diego, CA.*

The Cas9 protein is an emerging and powerful tool for engineering the genome in preclinical models for studying disease progression. Recent advances in CRISPR/Cas9 technology has enabled biomedical research to advance rapidly on multiple fronts, including target identification and on- and off-target mediated toxicities, as well as the implementation of genome editing for innovative therapeutic approaches. The first presentation will introduce novel advances in genome-wide high-throughput CRISPR/Cas9 screens to identify unique targets for RAS-driven cancers. The second talk will provide mechanistic insight into novel applications of the Cas9 protein for identification of on- and off-target toxicities during drug development of small molecules. The final talk will focus on the immunotoxicity of Cas9 in preclinical models with the goal of understanding implications on current genome-editing therapeutics. The collective content of this session will highlight both the advances and the challenges of CRISPR/Cas9 technologies during the different stages of drug development in a rapidly emerging field.

1727 Functional Genomic CRISPR/Cas9 Modifier Screens Unveil Novel Therapeutic Targets in the Context of RAS-Driven Cancers

J. Kwon. *Broad Institute, Cambridge, MA.* Sponsor: J. Willy

The mitogen-activated protein kinase (MAPK) pathway is a critical effector of oncogenic RAS signaling, and inhibition of this pathway may be a key component of effective combination treatment strategies. We performed genome-wide loss-of-function CRISPR-Cas9 modifier screens in the presence of the MEK1/2 inhibitor (MEKi) Trametinib in KRAS-mutant pancreatic and lung cancer cell lines. We utilized STARS algorithm, a predictive model of on-/off-target sgRNA activity, to identify genes and pathways that cooperate with MEK inhibition. While we observed significant heterogeneity in genetic modifiers of MEK inhibitor sensitivity across cell lines, several recurrent classes of synthetic lethal vulnerabilities emerged at the pathway level. Multiple members of the receptor tyrosine kinase (RTK)-RAS-MAPK signaling pathway recurrently scored as sensitizers to MEKi. In particular, we demonstrate that knockout or suppression of SHOC2, a positive regulator of MAPK signaling, specifically cooperated with MEK inhibition to impair proliferation of a broad range of RAS-driven cancer cells. The depletion of SHOC2 disrupts survival pathways triggered by feedback RTK signaling in response to MEK inhibition in RAS-driven cancers. Taken together, loss-of-function CRISPR modifier screens provide rich insights into common, dynamic networks involved in MEK inhibitor resistance across a panel of heterogeneous cell lines and nominate SHOC2 as a novel combination therapeutic target.

1728 Using CRISPR/Cas9 to Explore Mechanisms of Toxicity

J. Willy. *Vertex Pharmaceuticals Incorporated, San Diego, CA.*

Drug-induced liver injury (DILI) is a major contributor to the overall clinical occurrence of acute liver failure (ALF), often leading to early termination of clinical trials, post-marketing drug withdrawals, and the need for liver transplantation, and compound-specific causality is not always clear. Despite a recent pivot toward utilization of *in vitro* tools for early safety assessment, nonclinical safety studies are still utilized to predict clinical liabilities for new drugs. However, recent advancements in genome editing coupled with network-based approaches in toxicogenomics allow new insight to explore relationships from the molecular/cellular level to pathological changes occurring at the organ in preclinical studies. Here, we will focus on recent investigations utilizing an integrated systems biology tool kit consisting of CRISPR/Cas9 and toxicogenomics to reduce uncertainty for both adaptive and progressive changes in the liver during early safety assessment.

1729 Exploring the Toxicity of Cas9 Systems for *In Vivo* Editing in the Central Nervous System

E. Stahl. *University of California Berkeley, Berkeley, CA.* Sponsor: J. Willy

Adeno-associated viruses (AAVs) are promising vehicles for genetically delivering the Cas9 endonuclease and its single-guide RNA into cells. Although AAVs are efficient at transducing various types of cells, we hypothesize that durable expression of Cas9 might cause off-target editing or prolonged presentation of bacterial antigens on MHC molecules, alerting the immune system to the presence of foreign invaders. Cas9 also can be delivered nongenetically as a ribonucleoprotein (RNP), which is a pre-formed, potent, and short-lived effector complex. Cas9 itself does not have cell-penetrating ability and so was engineered to possess multiple Simian vacuolating virus 40 nuclear localization sequences (SV40-NLS), thereby inducing transient and efficient gene editing in post-mitotic neurons in adult mouse brains (Staahl et al. 2017, *Nat. Biotech.*). To assess the toxicity associated with Cas9 editing in the murine central nervous system, we designed a sgRNA to allow expression of a reporter in targeted cells after delivering the Cas9 effector complex by AAV or RNP. We then assessed both on-target and off-target editing by state-of-the-art NGS techniques and the presence of infiltrating immune subsets, inflammation, and a peripheral host response. Our results demonstrate the inherent toxicity associated with both off-target editing and the immune response given a specific delivery route, dosage, and vector, with the ultimate goal of understanding and improving the safe and therapeutic use of Cas9 as a gene editor.

1730 Bringing Advances in Risk Science into Regulatory Decisions: Applying Data-Driven Uncertainty Analysis

G. Woodall. *US EPA, Research Triangle Park, NC.*

Confidence is improved in human health risk assessments when variability is well characterized and uncertainties are based on objective, empirically derived observations (data) instead of defaults. Uncertainty analysis, although relevant for both exposure and toxicity, increasingly focuses on the toxicity values applied in human health risk assessment (NAS 2009). In this Workshop, we will present the state-of-the-science on methods for explicitly incorporating information and judgment in the derivation of toxicity values, and corresponding characterization and communication of risks. Many of these methods can now be applied given the datasets and tools currently available. Other methods highlight specific data gaps that can be prioritized to improve risk management decision-making. This Workshop will present perspectives from regulatory, industry, and academic risk assessment practitioners on systematic review, refined dose-response analysis, data-driven distributions of uncertainty factors, and leveraging human data in toxicity assessments. *The views expressed are those of the authors and do not represent the views or policies of the US EPA.*

1731 Interpreting Dose-Response Uncertainty in a Regulatory Environment: Relationship to Decision Context

K. Raffaele. *US EPA, Washington, DC.*

Risk assessors and decision-makers at the US Environmental Protection Agency (US EPA) strive to develop risk management options designed to be protective of the general population as well as potentially sensitive populations and life stages. However, specific regulatory decisions and the populations evaluated vary considerably based on the specific language of individual statutes. Over the years, US EPA has developed robust guidelines and procedures to support the hazard and dose-response assessments that are conducted in support of US EPA risk assessments. Ideally, a single hazard assessment paradigm can provide support for multiple decision contexts; however, available toxicity and dose-response databases vary considerably among different chemical substances (including types and reliability of available data, as well as dose-response estimation). In addition to uncertainty related to available toxicity data, uncertainty related to population characteristics and exposure evaluation also may vary according to the specific decision context, and needs to be considered. Given the differences in assessment goals, the impact of uncertainty on the decision also can be quite different. For example, the type of analysis (including estimates of uncertainty) that might be needed to support an emergency response is very different from that which will be useful in supporting a national population-based regulation. The utility of various types of uncertainty analyses will be discussed in the context of a range of regulatory decisions that must be made, including the trade-offs provided by different types of uncertainty information.

1732 Survey of Case Studies in Application of Approaches for Quantitative Evidence Integration and Uncertainty Analysis

C. Ring. *ToxStrategies Inc., Austin, TX.*

The increased use of evidence-based methodologies, such as systematic review, provide a platform for quantitative integration and uncertainty evaluation in risk assessment. Herein, we survey a number of such approaches as applied to the evidence base for dioxin and dioxin-like compounds. These include (1) meta-regression, to quantitatively integrate dose-response data from a set of *in vivo* animal studies of a single endpoint of interest; (2) a Bayesian approach to applying uncertainty factors, to quantify the uncertainties in extrapolating a point of departure (derived from dose-response data) to a health-protective reference dose; and (3) a Bayesian meta-analysis approach to quantitatively integrate a database of relative potency estimates for 28 dioxin-like compounds, including the effect of relative quality of each estimate. Challenges in the use of these techniques will be emphasized, including discussion of the influence of heterogeneity in the toxicology evidence base, limitations in data reporting, and communicating results of probabilistic analyses in a way that is meaningful for decision-making. Despite these challenges, these quantitative techniques provide enhanced opportunities to characterize risk and uncertainty that ultimately provide the risk assessor and risk manager with information well beyond that from a point-estimate toxicity value.

1733 A Probabilistic Approach for Dose-Response Assessment of Human Health Effects

T. Blessinger. *US EPA, Washington, DC.* Sponsor: G. Woodall

To address the growing need for a probabilistic approach for characterizing uncertainty in dose-response assessment, a Workgroup under the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) developed a step-by-step probabilistic approach for dose-response assessment of animal toxicology data. This approach derives health-based toxicity values (such as RfDs) or risk-specific doses probabilistically based on estimating a "target human dose" (HDMI). The HDMI can be calculated for a range of choices for the magnitude (M) of individual effect being protected against, the remaining incidence (I) of individuals with effects = M in the population, and the percent confidence in the HDMI estimate, thus allowing a risk manager to determine a target effect size, incidence, and confidence for a given decision. "Adjustments" to benchmark dose or other study results are made to account for uncertainty and variability. The approach allows the calculation of the dose that results in a preselected value of risk (i.e., a "risk-specific dose"). This approach can provide substantially more complete and transparent characterization of chemical hazards and support better-informed risk management decisions. *The views expressed are those of the author and do not necessarily represent the views or policies of the US EPA.*

1734 Globally Harmonized Guidance for Dose-Response Analysis and Derivation of Health-Based Guidance Values for Chemicals in Food. Part II—Intuitive Demonstrations

V. S. Bhat. *ToxStrategies Inc., Boston, MA.*

The World Health Organization (WHO) recently coordinated an international effort to update its guidance document on dose-response assessment for chemicals in foods, including additives, contaminants, natural toxicants, and residues of pesticides and veterinary drugs. This guidance also provides practical recommendations in deriving health-based guidance values, such as acceptable daily intake and acute reference dose, as the basis of establishing food safety regulations and safety standards in international food trade. Although the purpose of the guidance is related to the WHO food safety programs, the guidance reflects the latest expert thinking on dose-response assessment and benchmark dose (BMD) modeling, including some key areas of consensus among international experts, and so will be broadly applicable to areas beyond chemicals in food. Key motivators for the update were newly available modeling technology and the need for harmonization of the BMD approaches used by the US Environmental Protection Agency (US EPA) and the European Food Safety Authority (EFSA). Part I in this two-part Workshop includes presentations and demonstrations given by scientists who played essential roles in an international working group charged with drafting this harmonized guidance. Part I of the Workshop will focus on the basic elements and key updates in the new guidance, while Part II is designed as a practical demonstration session that builds on presentations in Part I. Part II will expand upon the concepts presented in Part I and provide nuts-and-bolts instruction on conducting BMD modeling with model averaging. The session will start with a brief introduction that outlines different BMD modeling platforms and highlights the need for harmonization. Then, two case studies, one with dichotomous data and the other with continuous data, will be demonstrated using both the US EPA BMDS software and EFSA PROAST software. Implications of key decisions in conducting the modeling also will be addressed. An additional case study will be provided to address more advanced topics, such as Bayesian prior selection and sensitivity analysis. Participants are encouraged, but not required, to bring their laptops with the latest version of BMDS and access to the web enabled, to get hands-on experience with the procedures and examples being demonstrated. The session will allow time for operational questions and discussion. Interested attendees can attend one or both Workshops.

1735 Benchmark Dose Modeling: Can We Bridge the Differences?

L. Haber. *TERA Center, University of Cincinnati, Cincinnati, OH.*

Benchmark dose (BMD) modeling has been generally accepted by many regulatory agencies to be the preferred approach to derive a point of departure (PoD) for risk assessment. In practice, BMD modeling can be performed on different platforms, including the BMDS software developed by the US EPA; the PROAST software that was first developed at the Netherlands National Institute for Public Health and the Environment (RIVM) and further advanced in collaboration with the European Food Safety Authority (EFSA); and Bayesian BMD (BBMD), a web-based software developed by Kan Shao, PhD, of Indiana University. In light of the need for consistency and transparency, this talk outlines key areas of differences in the practice and capabilities of these platforms, which serves as the introduction to the following interactive demonstrations that showcase the application of the harmonized WHO guideline.

1736 Benchmark Dose Analysis Using BMDS: An Interactive Demonstration

J. Davis. *US EPA, Cincinnati, OH.* Sponsor: V. Bhat

The presenter will analyze a dichotomous dataset and a continuous dataset separately, using the latest version of the US EPA BMDS software. The demonstration will be given in an easy-to-follow, step-by-step fashion that allows interactive participation from the audience. Emphasis will be given to the new developments that implement model averaging and key steps reflecting the new WHO guidance delineated in Part I. Note: if BMDS does not contain the model averaging feature for continuous responses at the time of the Workshop, a beta version will be used.

1737 Benchmark Dose Analysis Using PROAST: An Interactive Demonstration

J. Cortiñas Abrahantes. *European Food Safety Authority, Parma, Italy.* Sponsor: V. Bhat

The presenter will analyze the same dichotomous and continuous datasets used in the previous BMDS demonstration, using the latest version of the EFSA/RIVM PROAST package. The demonstration will be given in an easy-to-follow and step-by-step fashion, allowing interactive participation from the audience. Emphasis will be given to the application of model averaging and key steps reflecting the WHO guidance delineated in Part I, with additional commentary on how harmonization can be achieved in two different software packages.

1738 Boundary Cases for Bayesian Benchmark Dose Analysis

M. Wheeler. *NIOSH, Cincinnati, OH.* Sponsor: V. Bhat

In this demonstration, a case study on unanticipated results when applying Bayesian methods to dose-response analyses and benchmark dose estimation is studied. This presentation will discuss advanced considerations in BMD modeling such as model boundary conditions, priors, and flexibility. Specifically, this talk will investigate a dataset where traditional maximum likelihood methods produce unstable estimates and noninformative Bayesian analysis produces results that are not intuitive. The focus will be on the impact of prior selection on results and the importance of conducting sensitivity analysis. Means for integrating toxicological knowledge into priors for Bayesian approaches in BMD analyses will be discussed.

1739 Single Cell Applications in Mechanistic Toxicology

C. Smith. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Investigations into the mechanism of action of chemicals, pharmaceuticals, and contaminants typically rely on measurements of gene and protein expression. A change in expression of a gene, protein, or functionally related groups would indicate perturbation of suspect signaling pathways. These measurements are typically performed in whole tissues or homogenous populations of cells in culture. These "bulk" analyses do not consider the heterogeneity of cell types within a given tissue and thus provide a nonspecific average across cell populations, which reduces the specificity and sensitivity of the measurement. Different cell types exhibit variable responses to toxicant exposures that may be missed when analyzing bulk samples. For example, traditional bulk analyses cannot decipher responses in low abundant cells, which can be diluted by changes in more abundant cell types, and do not capture opposing signals in multiple cell types. To address these issues, single cell analytical tools have been developed that probe alterations in the genome, transcriptome, proteome, and metabolome of individual cells. While these methods are gaining popularity in the fields of immunology and cancer biology, among others, they have been minimally used to characterize target organ toxicities or to understand the mechanisms of action of toxicants. The purpose of this Workshop is to provide the audience an overview of the field of single cell biology through case studies of mechanistic investigations into toxicological responses in multiple systems using various single cell analytical methods. The first presentation will focus on antibody-based methods used to measure protein expression in single cells, including flow cytometry and single cell western blotting. The next presentation will focus on the use of single cell RNA sequencing (scRNA-seq) data to train a deconvolution algorithm, CIBERSORT, to estimate population size shifts from bulk RNA-seq data. The last presentation will introduce the single-cell amalgamation via latent semantic analysis (SALSA) workflow for cross-specimen integration of scRNA-seq data that is used to extract reliable exposure-induced gene expression changes from scRNA-seq data by reducing noise and addressing issues related to the sparsity of single cell data. This presentation also will complement scRNA-seq data with single cell proteomic analysis of cell surface proteins using single cell mass cytometry. Overall, this Workshop will introduce attendees to multiple single cell analytical techniques that provide a deeper view of the underlying biology driving adverse toxicological responses in diverse systems and will allow ample interaction between participants.

1740 Application of Single Cell Proteomic Analyses to Identify Pharmacological Mechanism of Action of a Novel Nitrated Fatty Acid in Acute Lung Injury

C. Smith, M. Wilkinson, A. Murray, E. Abramova, C. Guo, and A. Gow. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

The lung is a complex organ composed of over 40 cell types that are differentially targeted by contaminant exposures and respond in an intricate and coordinated manner to repair the lung after injury. Our laboratory utilizes multiparameter flow cytometry and single cell western blotting to elucidate mechanisms of action of toxicants and to identify potential targets for therapeutic intervention. This study examined the efficacy of nitrated-oleic acid (OANO2) in mitigating acute lung inflammation in mice intratracheally exposed to bleomycin (ITB). ITB treatment significantly increased total protein in bronchoalveolar lavage (BAL) (413±45.7 µg/mL) compared with controls (67±32.2 µg/mL), which was reduced by OANO2 (355±54.3 µg/mL). Histological analysis revealed cell infiltration and tissue injury in ITB mice that was reduced by OANO2. Flow cytometry of cells recovered from BAL demonstrated loss of Siglec-F+F4/80+CD45+ alveolar macrophages (37±3.6%; %CD45+ cells) relative to controls (95±3.3%; %CD45+ cells), and an increase in nonresident macrophages (48±4.1%; %CD45+ cells) compared with controls (4±3.8%) that was decreased by OANO2 (34±3.8%; %CD45+ cells). Mesenchymal cells (CD31-CD45-Sca-1+) isolated from lung digest demonstrated an increase in CD44 and CD90 expression in response to ITB (3±0.94 versus 23±1.0%; 43±2.3 versus 74±2.6%; %CD45- cells), suggesting an increase in fibrotic and proliferative potential, respectively, which was significantly reduced by OANO2 (19±0.9%; 70±2.3%; %CD45- cells). Single cell analysis of mesenchymal cells by singe cell western blot revealed ITB-induced expression of the profibrotic protein ZEB1; coadministration of OANO2 reduced the percentage of ZEB1 expressing cells. Single cell western blotting of the proinflammatory marker, HMGB1, in CD45+ cells revealed three populations of cells, cells with no HMGB1, low-expressing cells, and high-expressing cells. ITB resulted in an increase in high-expressing cells and loss of low-expressing cells. While coadministration of OANO2 did not abolish HMGB1 high-expressing cells, it increased the number of low-expressing cells. Overall, these findings suggest that treatment with OANO2 mitigates ITB-mediated proinflammatory cellular activation by altering resident cell function and promoting resolution of inflammation and highlight biphasic responses in CD45+ cells.

1741 Deconvolution of Bulk RNA-Seq Data Using Archived Single Cell RNA-Seq Data to Determine the Effects of Toxicants on Cell Population Sizes

D. Ruden, and J. Isherwood. *Wayne State University, Detroit, MI.* Sponsor: C. Smith

Lead has been used in a variety of products and industries; it is pervasive in the environment, and we are exposed to it through a variety of sources, like water from lead plumbing and dust. The developing nervous systems of children are most significantly affected by lead exposure, resulting in cognitive dysfunction and neurobehavioral deficits—for instance, learning and memory problems and lower IQ. This neurotoxicity is a result of the oxidative damage inflicted by lead, as well as ion mimicry, where lead can replace certain ions, like zinc and calcium. In our laboratory, we were interested in the effects of lead exposure on brain cell populations. To investigate this, we utilized publicly available *Drosophila* brain single cell sequencing data, from our lab and other labs, and bulk RNA-seq data of *Drosophila* heads +/- lead exposure generated in our lab. To identify the cell types most affected by lead exposure in the brain, we adapted the cell type deconvolution method CIBERSORT that was originally developed to identify blood cell type levels from bulk RNA-seq data. The single cell sequencing data from *Drosophila* brains identified 37 clusters of brain cell types, and the cluster-specific gene expression profiles were used as the input reference data for deconvolution. The results were proportions of each of the 37 cell type clusters for each of the lead exposed and control *Drosophila* head samples. Wilcoxon tests were performed, comparing the cell type cluster proportions between the lead exposed and control samples, identifying three significantly different cluster proportions: the a/ß lobe and a′/ß′ lobe of the mushroom body, and a group of dopaminergic neurons. Interestingly, the clusters corresponding to the a/ß lobe of the mushroom body, involved in long-term memory, and the dopaminergic neurons, involved in learning, both showed significant decreases in proportion comparing control with lead-exposed samples. In conclusion, we used single cell RNA-seq data to identify two neuronal cell types that decrease in number after developmental lead exposure. Further characterization of these two neuronal cell types are in progress.

1742 Peripheral Blood Monocytes of Human Smokers Exhibit Differences in M1 Macrophage-Associated Transcriptional Signatures Resolved by Single Cell RNA Sequencing

O. Lozoya, S. Martos, M. Campbell, M. Iannone, G. Pittman, and D. Bell. *NIEHS, Research Triangle Park, NC.* Sponsor: C. Smith

Smoking alters the DNA methylation and transcriptional profile in peripheral blood mononuclear cells (PBMCs). Given the large inter-individual variation that characterize scRNA-seq technologies, large cell numbers are preferred to explore the effects on targeted PBMC subpopulations, yet limited monocyte densities in PBMCs preclude confident analysis of tobacco-induced effects. Also, current scRNA-seq analytical pipelines lack resolution metrics to isolate exposure-induced transcriptional differences beyond cell-type identification across biological replicates. To circumvent these roadblocks, we exploited the advantages of the single-cell amalgamation via latent semantic analysis (SALSA) workflow for cross-specimen integration of sc-RNAseq data (doi: 10.1101/551762). SALSA is a multivariate scRNA-seq analysis method that optimizes discovery of reproducible gene expression changes from ultra-sparse single cell data across samples, statistical groups, and scRNA-seq platforms. First, we profiled >32,000 pan-monocyte enriched PBMCs isolated from multiple smoking and nonsmoking human donors (N = 4 independent specimens each group; median: 4,000 cells/donor; average: 2,800 net UMI/cell). Then, we implemented SALSA by anchoring multivariate analysis on genes that drove within-donor clustering, were shared across donors within smokers or nonsmokers, and showed statistically reproducible effect sizes and statistically significant multivariate comparisons. Altogether, our analysis revealed a minimal subset of 833 transcriptional profiling candidates among seven monocyte subpopulations, with transcriptional signature for M1 polarization downregulated in smokers. Ongoing work also includes mass cytometry characterization of equivalent pan-monocyte fractions from these donors revealing differential expression signatures of cell surface marker proteins. In sum, use of a stratified integration approach through SALSA for scRNA-seq analysis was able to ameliorate individual-specific batch effects and inter-group false-positive rates. This was accomplished while extracting the dominant sources of intra- and inter-individual variations stemming from underlying monocyte subpopulations, permitting integration with protein-level observations by mass cytometry.

1743 The More, the Better? Single Biomarkers, Panels, and/or Composite Biomarkers to Characterize Organ Injury

P. Devine. *Novartis Institute for BioMedical Research, Cambridge, MA.*

Biomarkers are critically important for disease diagnosis and for safety and efficacy evaluations during drug development. A number of traditional biomarkers, along with more recent diagnostic and exploratory biomarkers, are utilized in standard clinical pathology assessments. The Critical Path Institute Predictive Safety Testing Consortium, industry, and academic researchers have performed prospective preclinical and clinical studies on newer biomarkers that are being developed and characterized to improve differential diagnoses, especially identification of specific types of organ injury and injury or toxicity in the presence of confounding disease conditions. Examples of traditional biomarkers include amylase and lipase for injury to the exocrine pancreas, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) for hepatocyte injury, creatine kinase (CK) for skeletal muscle injury, and urinary protein for kidney injury. Specific situations can confound the interpretation of these traditional biomarkers. One example is that muscle injury or intense exercise can alter biomarkers of liver injury like ALT. In addition, another traditional liver injury biomarker, γ-glutamyltransferase (GGT), was found to be associated with cardiac damage, altering the once indisputable correlation of GGT and ALT/AST with liver injury. Greater confidence in interpretation of biomarkers may be gained by examining more than one endpoint at a time; however, this requires larger sample volumes, higher costs, and greater efforts. Furthermore, increased numbers of endpoints may lead to confusion and increased risk of false positives. More recently, panels of biomarkers have been tested for their composite diagnostic predictability. Strategies for utilization and implementation of new and exploratory biomarkers into current practices and guidelines are needed. This Workshop will describe the latest recommendations on how exploratory biomarkers should be interpreted and implemented within the context of traditional biomarkers and panels of biomarkers and whether composite biomarker readouts may improve diagnoses. Three speakers will discuss the latest in vascular injury, skeletal muscle, and kidney biomarkers and the various ways in which these biomarkers can be utilized. The session will conclude with an interactive panel discussion, driven by questions from the audience. Together, these talks will examine how to incorporate new biomarkers into current panels and strategies as they are explored, characterized, and eventually validated.

1744 Lessons Learned from the Qualification of Vascular Injury Biomarkers

J. Brumm. *Genentech Inc., South San Francisco, CA.* Sponsor: P. Devine

This talk will review and summarize the candidate biomarker identified by the Drug-Induced Vascular Injury (DIVI) Working Group (WG) of the Predictive Safety Testing Consortium (PSTC). DIVI is a poorly monitorable toxicity for which there are no specific standards to measure early onset or recovery. The selection of candidate biomarkers by the DIVI WG was based partly on the nature and breadth of the biomarker panel biology including response over duration of injury, across vascular compartments and shared response/morphologic outcome to injury regardless of pathogenesis/mechanism of the vascular insult. It is anticipated that the signature of the biomarker panel may vary according to pathogenesis, but collectively in a univariate or multivariate/combination approach, the biomarkers will be able to detect early end-organ vascular injury. To achieve specificity to the vascular system while maintaining sensitivity, we plan to have a biomarker panel with minimally at least one biomarker from each compartment (i.e., endothelial, smooth muscle, inflammation). We will review the approach that was taken to conduct and analyze the preclinical studies and highlight lessons learned with regard to bioanalytical methods and data analysis approaches.

1745 Clinical Biomarkers for Skeletal Muscle Injury and Neuromuscular Diseases

W. E. Glaab. *Merck & Co. Inc., West Point, PA.*

Skeletal muscle (SKM) injury has been reported as an adverse event as a result of a number of medications, including statins and fibrates. Conventional markers, such as creatine kinase (CK) and aspartate aminotransferase (AST), lack the sensitivity and specificity to detect SKM injury. New biomarkers of SKM injury would be useful as effective drug development tools not only to monitor toxicity and reversibility in nonclinical studies, but also to assess the safety of compounds in early clinical development. Yet another potential benefit of new SKM injury/degeneration biomarkers is that they can be used to monitor muscular disease progression and therefore be indicators of drug efficacy in clinical trials of new therapies for neuromuscular disorders. Information presented will highlight work of the Predictive Safety Testing Consortium's Skeletal Muscle Working Group and summarize the nonclinical data generated through this collaborative effort. Such data demonstrate the performance of four SKM biomarkers of drug-induced SKM injury—Skeletal troponin I (sTnI), Myosin light chain 3 (Myl3), Fatty-acid binding protein 3 (Fabp3), and Creatine kinase muscle type (Ckm). These nonclinical data were submitted to the US Food and Drug Administration and European Medicines Agency and sTnI, Myl3, Fabp3, and Ckm were endorsed with Letters of Support from both Agencies. Translation to clinical settings also will be discussed, including baseline assessment in healthy volunteers, patients with drug-induced injury, or muscular dystrophies (e.g., Duchenne muscular dystrophy, myotonic dystrophy type 1, and spinal muscular atrophy), with consideration of single biomarker versus multiple biomarker performance.

1746 Composite Measures of Multiple Urine Biomarkers as Early Drug Development Tools to Detect Renal Injury Response

R. Mogg. *Bill & Melinda Gates Medical Research Institute, Philadelphia, PA.* Sponsor: P. Devine

The Kidney Safety Project (KSP), a collaborative effort of the Foundation for the National Institutes of Health (FNIH) and Critical Path Institute (C-Path) Predictive Safety Testing Consortium, has proposed the potential use of two different composite measures in early drug development safety monitoring, each derived from a panel of urine biomarkers under a different context of use. The first composite measure, which received a positive qualification determination letter in August 2018, is to be used in early normal healthy volunteer trials to monitor group-level increases above a threshold to indicate the potential presence of renal tubular injury. The second composite measure, which is currently being prospectively evaluated for submission to regulators, also is to be used in early drug development trials, but to monitor the presence of renal tubular injury in individual patients. This presentation will detail the methods utilized to develop the two different composite measures to assess renal tubular injury and the benefits of using multiple biomarkers as opposed to a single biomarker in this context, while highlighting the limitations of the datasets that were available for the derivations and the regulatory comments received throughout the program.

1747 Variety Is the Life of Spice: The Clinical Toxicology of Synthetic Cannabinoids

S. Bradberry. *National Poisons Information Service (Birmingham Unit), Birmingham, United Kingdom.*

In the last 10 years, the abuse of synthetic cannabinoid receptor agonists (SCRAs) has caused unprecedented physical and psychological ill health among users, with an associated substantial burden on health and social care providers. Initially identified in the early 1980s, the repertoire of synthetic analogues has expanded rapidly to several hundred compounds. SCRA molecular structure consists of four components—"core," "tail," "linker," and "linked" groups—each with numerous potential analogues. Structural diversity presents challenges for nomenclature, with several systems in use, including serial designations generated by laboratories, names used by recreational users, and an alphanumeric systematic nomenclature. When applied correctly, the latter can effectively communicate detailed information about the structure and potential toxicity of these compounds. Unfortunately, this system has been inconsistently applied, and there remains a lack of international consensus on its proper usage. SCRAs are typically full agonists at cannabinoid receptors (CB1 and CB2) with potency up to one hundred times that of naturally occurring tetrahydrocannabinol. They also have the potential for many downstream signaling effects because of coupling of CB1 and CB2 to a variety of signal transduction mechanisms, including inhibition of adenyl cyclase and activation of mitogen-activated protein kinase. There is particular concern regarding the psychotic effects of these compounds, with evidence that they can affect the functioning of neurotransmitter systems other than that of endocannabinoids, including dopamine, serotonin, and glutamate, neurotransmitters known to be implicated in the pathophysiology of psychosis. Synthetic cannabinoids are typically sprayed onto plant material to be smoked. Their clinical effects are diverse and unpredictable. Central nervous system toxicity predominates with coma, seizures, extreme agitation, and psychosis. Other complications include stroke, myocardial infarction, acute kidney injury, rhabdomyolysis, and hyperthermia. Fatalities have occurred. Management is focussed on symptomatic and supportive measures, with an emphasis on maintaining staff and patient safety through conscious sedation. Poor understanding of the mechanisms of toxicity, limited dose-response data, and problems with real-time analytical confirmation contribute to the clinical challenge these patients bring to the emergency department. Where available, Ultra-Performance Liquid Chromatography with Time of Flight Mass Spectrometry (UPLC-MS/TOF) offers accurate, flexible, and specific analysis. In specialist units, this technology has been utilized to develop a vigilance system that identifies those cannabinoids in current use by analysis of drugs donated by or confiscated from users. The results are used to create a targeted, rapid turnaround drug screen pertinent to the particular cannabinoid analogues in circulation at a given time. Latest challenges surrounding SCRAs include the potential adverse health effects of long-term use, with the associated risks of addiction to and subsequent withdrawal from synthetic cannabinoids as yet inadequately clarified. Legislation aimed at limiting availability of these drugs has been implemented in several counties with questionable success. There remains a global need to focus not only on law enforcement but also on education, harm reduction, and prevention.

1748 Synthetic Cannabinoids: Chemistry, Nomenclature, and Mechanisms

S. Hill. *National Poisons Information Service, Newcastle, United Kingdom.*
Sponsor: S. Bradberry

Originally developed for legitimate scientific research, the repertoire of synthetic cannabinoids for recreational use has expanded rapidly in recent years, with at least 180 analytically confirmed compounds as of January 2019. The molecular structure of synthetic cannabinoids consists of four components: "core," "tail," "linker," and "linked" groups. Their structural complexity offers multiple opportunities for chemical modification to evade drug control legislation (itself based on chemical structure), explaining the large numbers of individual chemicals that have been developed. The structural complexity of synthetic cannabinoids presents challenges for nomenclature, with several systems in use, including serial designations generated by laboratories, names used by recreational users, and an alphanumeric systematic nomenclature that is increasingly being referenced in published literature and the recreational market. When applied correctly, the alphanumeric nomenclature can effectively communicate detailed information about the structure, and potential toxicity, of these compounds. Unfortunately, this system has been inconsistently applied, and there remains a lack of international consensus on its proper usage. Synthetic cannabinoids are typically full agonists at cannabinoid receptors, with potency up to one hundred times that of naturally occurring tetrahydrocannabinol. Moreover, they have numerous metabolites with diverse activity profiles at cannabinoid receptors (CB1 and CB2). They also have the potential for many downstream signaling effects because of coupling of CB1 and CB2 to a variety of signal transduction mechanisms, including inhibition of adenyl cyclase and activation of mitogen-activated protein kinase. There is particular concern regarding the psychotic effects of these compounds, with evidence that they can affect the functioning of neurotransmitter systems other than that of endocannabinoids, including dopamine, serotonin, and glutamate, neurotransmitters known to be implicated in the pathophysiology of psychosis. This presentation will discuss the chemical structure of synthetic cannabinoids and describe the different nomenclature used to identify individual compounds, linking where possible structure to activity.

1749 Clinical Presentation of Synthetic Cannabinoid Exposures and the US Experience

J. Brent. *University of Colorado School of Medicine and School of Public Health, Denver, CO.*

In 2014, the Toxicology Investigators Consortium noted a sharp uptick in cases of toxicity from synthetic cannabinoids (SC). The severity of toxicity ranged from mild to severe. Of 456 patients, the most common features of toxicity were neuropsychiatric, primarily manifested as agitation, delirium/toxic psychosis (41%), and central nervous system depression/coma (24%). Seizures (17%) and hallucinations (7%) occurred less frequently. 28% of cases presented with severe vital sign abnormalities: 12% tachycardia (>140 beats per minute [BPM]), 6% bradycardia (<50 BPM). 5% had rhabdomyolysis, and 3% had acute kidney injury. The death rate from synthetic cannabinoid-only exposures was 1.2%. In the US, synthetic cannabinoids are used by spraying onto plant material and smoked, added to food/beverages, or placed in electronic nicotine delivery devices. Epidemiologic data on exposures is hampered by the lack of analytical confirmation in most cases. However, the peak year for cases of presumed SC exposures reported to US poison centers was 2015. Importantly, the possibility of contamination of SC products with other biologically active molecules has been a major epidemiologic concern. For example, a recent outbreak of SC-related coagulopathy occurred due to contamination with the anticoagulant brodifacoum.

1750 Synthetic Cannabinoid Abuse: Diagnosis and Management

S. M. Bradberry. *National Poisons Information Service (Birmingham Unit), Birmingham, United Kingdom.*

While patients with mild features of acute synthetic cannabinoid intoxication may self-present with a clear history of exposure, it is not unusual for emergency physicians to be faced with an aggressive, incoherent individual from whom a reliable history is not forthcoming. In these cases, the initial diagnosis is based on the presence of classic clinical features, sometimes accompanied by circumstantial evidence or collateral information from family or friends. Analytical confirmation of synthetic cannabinoid use is not widely available since they are not detected by classic immunoassay-based screens. Diagnostic uncertainty can result in patients undergoing expensive and/or invasive investigations as part of their clinical workup. Even if more specific analysis is available, the changing pattern of synthetic cannabinoids in circulation can result in false-negative results. Analytical toxicovigilance systems utilizing Ultra-Performance Liquid Chromatography with Time of Flight Mass Spectrometry (UPLC-MS/TOF) have enabled accurate identification of "unknown" toxins and flexible, targeted modification of "routine" screening repertoires to match changing clinical need. Management of the acute phase of poisoning is focused on symptomatic and supportive measures, with an emphasis on maintaining patient and staff safety through conscious sedation. In extreme cases, paralysis and mechanical ventilation are required. Most patients make an uneventful recovery, though life-changing, permanent sequelae are increasingly documented, and fatalities have occurred. There is evidence that the latter is underreported, particularly among the homeless population, in whom use of synthetic cannabinoids is a particular public health challenge. There also is increasing concern regarding the potential adverse health effects of long-term use, with the associated risks of addiction to and subsequent withdrawal from synthetic cannabinoids as yet inadequately clarified. Legislation aimed at limiting availability of these drugs has been implemented in several counties with questionable success. There remains a global need to focus not only on law enforcement but also on education, harm reduction, and prevention.

1751 Cellular Recovery and Resilience: A New Perspective on Toxicity Testing

L. Smirnova. *Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

The move from whole animal-based toxicology tests to human cell-based assays has witnessed a renewed focus on toxicological mechanisms. Technological developments, including 'omic technologies, have enabled an unprecedented understanding of how cells deal with chemical insult, which is often by altering transcriptional programs to initiate specific stress response pathways. Currently, many *in vitro* toxicity studies mainly assess acute susceptibilities of cells and tissues but ignore long-term, low-dose, epidemiologically relevant exposures to chemicals, which could lead to disease manifestations. The concept of cellular resilience, in contrast, suggests studying how cells can cope with stress by activating pathways of defense, which lead to recovery and resistance (tolerance) against subsequent hits. The first presentation will demonstrate how such defense mechanisms could help us to identify vulnerable individuals or irreversible adverse outcomes that manifest as progressive diseases. Pathways such as Nrf2 (oxidative stress), p53 (DNA damage), HIF-1 alpha (hypoxia), and Unfolded Protein Response (proteotoxicity) are among the most frequent ones to provide the cell with some resistance to the initiating injury. Such pathways are by definition protective, and their activation can be used to identify the molecular initiating event and the extent of the impact. The next presentation will continue the discussion about adaptation, recovery, and resilience, with the focus on neurodegeneration. The third presentation will demonstrate how the field of cellular recovery and resilience can be addressed in a high-throughput manner by introducing the Toxmatrix, a chemical genomics method that can identify chemicals, and pathway perturbations, that protect or sensitize cells to a toxicant. Characterization of these pathways also enables us to connect dose-responses to irreversible adverse events, as detailed in adverse outcome pathways. Quantitative high-throughput screening approaches and transcriptomic biomarkers have been used to identify activation of the stress pathways. Cell co-culture and 3D models also are being developed to understand chronic adaptations such as cardiac hypertrophy, Epithelial-to-Mesenchyme Transition (EMT), neurodegeneration, and fibrosis. The fourth talk will present recent work on "tipping points" between adaptation and adversity based on high content imaging data from the ToxCast project. Tipping points are another approach for quantifying cellular resilience as the concentration and/or exposure duration beyond which a cell is unable to recover. The discussion on recovery and resilience will be completed by in the final presentation, which will introduce the methods of how to measure biomarkers of resilience. Biomarkers of specific stress phenotypes and tissue dedifferentiation also may be identified for tipping points that enable us to quantify the exposure threshold that triggers an irreversible adverse effect. Taken together, intoxicated cells may differ less in how they are hit than in how they resist stress and recover or adapt to the new homeostasis to become more resilient. Defining these mechanisms of resilience will enable us to generate better *in vitro* assays and will inform both animal and clinical studies. This Symposium will address both resilience and recovery after injury. The main findings on cellular recovery and resilience from the five presentations and future directions will be wrapped up in the summary talk.

1752 Lessons Learned from Repeat-Dose Toxicity and Recovery after Exposure

P. Jennings. *Vrije University Amsterdam, Amsterdam, Netherlands.*
Sponsor: L. Smirnova

While focused, well-carried-out, acute *in vitro* experiments can be very helpful for determining molecular initiating events, it might be expected that longer repeat exposures and recovery experiments would be required to predict effects for chronic exposures *in vivo*. Unfortunately, such experiments are rare. In this talk, specific examples of recovery and repeat dose will be presented, where stress responses, tissue-specific biomarkers, and kinetics are integrated. A specific example is that of differentiated human renal proximal tubule cells (RPTEC/TERT1 cell line), which were treated for five days with cyclosporine A (CsA), allowed to recover for eight days, and treated again for another five days. Transcriptomics, metabolomics, CsA-induced cyclophilin secretion, morphology, and CsA kinetics were measured over treatment and recovery periods. The second treatment was similar for many of the endpoints but had a dampened transcriptomics signal and CsA-induced glycolysis and CsA-induced acetate production. Since the biokinetic update of CsA was the same, this was unrelated to alterations in extrusion or metabolism genes. The value of repeat dose and recovery to add to the predictive weight of *in vitro* experiments to human safety assessment will be critically analyzed.

1753 The Cellular Resilience Concept: Application to Chemical-Induced Parkinson's Disease Model

L. Smirnova. *Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

Mechanisms of resilience are being examined in a repeat-dose neurotoxicology paradigm. In Parkinson's disease, the systemic effect of mitochondrial complex I inhibition by a chemical such as rotenone leads to selective degeneration of dopaminergic neurons but not other neurons. We hypothesized that these cells are less resilient and cannot cope with the same stress as other neurons and therefore are targets for neurodegeneration. We analyzed acute and delayed response of 3D dopaminergic neuronal model (3D LUHMES) to low-dose rotenone to identify early responses versus defense; recovery versus adverse outcome. We demonstrated that 3D LUHMES could functionally recover from the low-dose exposure to rotenone, with some molecular scar remaining. The recovered cells maintained a higher energy production level and higher metabolic capacity and were more resilient to the second hit. However, the fully functionality of those resilient cells was not restored. Intervention and repair of perturbed molecular pathways (scars) can stimulate functional resilience. For example, overexpression of neuroprotective microRNA-7 may help cells to stay resilient in the long-term perspective. These processes illustrate on a molecular level how chemical exposure alters cellular responsiveness, including the induction of protective means leading to resilience, but also molecular scars possibly associated with late hazard manifestations.

1754 Probing Resilience Mechanisms by Chemical Combination Screening

D. Gerhold. *NIH/NCATS, Bethesda, MD.*

Extending the neurodegenerative toxicology paradigm, we developed a chemical genomics method, the "Toxmatrix," to identify mechanisms of toxicity and resilience. Using this method, cultured cells were treated with a toxicant at 10 concentrations, and additionally pre-treated with each of 70 modulator chemicals, to identify modulators that shifted the IC_{50} for cell killing. By screening all combinations of 32 toxicants by 70 modulators, we identified 20 toxicant-modulator pairwise interactions, including 14 pairs that showed protection or sensitization in multiple neuronal cell lines. For example, the modulator buthionine sulphoximine (BSO) sensitized neuronal cell lines to 6-hydroxydopamine by inhibiting glutathione synthesis. This implies that glutathione synthesis is a resilience pathway, protecting neurons from 6-hydroxydopamine. Conversely, chelator DMPS protected three neuronal cell lines from 6-hydroxydopamine and four other toxicants. DMPS acts by chelating metal ions, implying that these five toxicants disrupt metal distribution pathway(s). Since each modulator was chosen to inhibit or activate a single annotated target in the cell, the modulator-toxicant interaction provides information about the toxicant's potential mechanism—hence, pathways of toxicity or cellular resilience. Surprisingly, several modulators were found that sensitized or protected neuronal cells from multiple diverse toxicants, identifying susceptible pathways that may be characteristic of neurons.

1755 Toxicological Tipping Points: Using High-Content Imaging Data from ToxCast

I. Shah. *US EPA/NCCT, Research Triangle Park, NC.*

This talk will explore resilience mechanisms using high content imaging (HCI), recognizing that a "tipping point" dose may be reached where adaptive responses yield to adverse events. We have studied the effects of 88 chemicals in primary rat hepatocytes using HCI to measure dose- and time-dependent perturbations in endoplasmic reticulum stress, mitochondrial function, lysosomal mass, steatosis, apoptosis, DNA texture, nuclear size, and cell number. A new computational model is proposed to describe the dynamic response of the system as cell-state trajectories based on multidimensional HCI data streams. Cell-state trajectories produced by multiple concentrations chemicals showed resilience of the primary rat hepatocyte system in many cases; however, we also found "tipping points" in system recovery. Further analysis of trajectories identified dose-dependent transitions, or critical points, in system recovery. We believe that HCI can be used to reconstruct cell state trajectories and provide insights into adaptation and resilience.

1756 Sensor-Based Assays to Measure Markers of Cellular Resilience in Real Time

C. Agu. *INanoBio, Mountain View, CA.* Sponsor: L. Smirnova

This talk will present a flexible technology for detection of cellular stresses and examples for early detection of such alarm signals. The ability to measure and study the molecular causes and phenotypic consequences of different levels of cell resilience requires the availability of tools that can detect and quantitatively measure differences in cellular resilience in real time in response to carefully controlled perturbations. We report a novel nano-sensor platform capable of *in vitro* label free measurement of markers of cellular stress and resilience (e.g., exosomal heat shock proteins) released by stressed cells in real time. Results from our nano-sensor-based real-time cell resilience assays will be presented, and their utility in investigating cell stress and resilience pathways will be discussed. The overarching goal is to scale the sensor array platform to ultra-high-throughput real-time assay of tens of thousands of single cells, simultaneously. When fully developed, this cell resilience assay platform will expand our understanding of individual differences in response to a broad spectrum of physiological stressors with the goal of promoting the development of novel therapies and interventions to preserve or enhance resiliency and promote healthy living.

1757 Cyanide as a Chemical Threat: Finding Better Antidotes

D. Jett. *NIH/NINDS, Bethesda, MD.*

Cyanide is a well-known industrial chemical that also has been used as a deadly poison throughout modern history and antiquity. Several cyanide-containing compounds, including hydrogen cyanide, potassium cyanide, and cyanogen chloride, are highly toxic and considered a threat to public health. They are considered chemical threats because of potential use by terrorists and large-scale industrial accidents like the one that occurred in Bhopal in 1984. Cyanide disrupts cellular oxidative phosphorylation via the inhibition of cytochrome c oxidase. Cyanide also inhibits several other enzymes, induces oxidative stress, and increases cellular calcium. Mitochondrial toxicity and other effects lead to death, usually from cardiopulmonary paralysis. The central nervous system (CNS) also is vulnerable to cyanide toxicity, evidenced by the nonlethal chronic effects that are usually related to deficits in motor neuron functionality, like those reported after consuming cyanogenic cassava root (Konzo disease) and the parkinsonism reported in survivors of cyanide poisoning. The wealth of knowledge gained from decades of research on the mechanisms of cyanide toxicity has resulted in the development of effective therapeutics for individual exposures including the recently US Food and Drug Administration (US FDA)-approved drug Cyanokit. However, the unmet need discussed in this session is the lack of safe and effective therapeutics for treating many individuals at once after deliberate or accidental release. United States federal agencies such as the Departments of Health and Human Services and Homeland Security have identified a need for antidotes that have better utility in these mass causality events. The currently available antidotes are effective and relatively easy to use by trained professionals to treat individual poisonings. However, they can require reconstitution before administration, and all available US FDA-approved cyanide antidotes must be delivered by intravenous injection, which is difficult and time-consuming. These and other features of current antidotes may not be ideal for rapid treatment of many individuals at once. This session will describe the unmet need for better cyanide antidotes during mass causality events and provide specific examples of current innovative research on the discovery of new lead compounds and the development of drug candidates.

1758 Introduction and Overview of the Unmet Need in Cyanide Research

D. A. Jett. *NIH/NINDS, Bethesda, MD.*

There are two US FDA-approved cyanide antidotes currently available: Cyanokit (hydroxo-cobalamin) and Nithiodote (sodium nitrite and sodium thiosulfate). Additionally, there is a third treatment, amyl nitrite, which has traditionally been packaged with sodium nitrite and sodium thiosulfate as the Cyanide Antidote Kit. Cyanokit and Nithiodote require intravenous administration by trained personnel. While these antidotes are effective for single cyanide poisonings, better antidotes for mass casualty events is a current unmet need. Some of the characteristics new and improved cyanide antidotes should have include that they (1) can be administered by minimally trained individuals, possibly with intramuscular autoinjectors or oral, intranasal, or inhalational administration; (2) have a rapid onset and long duration of efficacy; (3) possess efficacy for all forms of cyanide and routes of cyanide exposure; (4) have a ready-to-use antidote not requiring preparation in the field; (5) are stable for storage of many doses; and (6) are cost-effective. Current research on better cyanide antidotes includes basic mechanistic research to discover therapeutics targets, through preparing drug candidates for advanced development and ultimate US FDA approval and licensure. Cyanide research can be categorized as either cyanide-based or host-based. Cyanide-based research includes agents that bind cyanide and remove it from circulation, while host-based research focuses on the detoxification of cyanide by increasing its metabolism and elimination, or by direct interaction with targets of cyanide and other mechanisms that prevent or ameliorate acute and chronic toxicity. Special consideration is being given to alternate routes of administration and/or dosing regimens for new or already US FDA-approved therapies that would be safer, more effective, or easier to administer during a mass casualty scenario. Specific subpopulations (e.g., pediatric and pregnant) that are more vulnerable also are a priority for civilian exposures. Finally, clinical efficacy studies are rare because of the obvious ethical challenges; however, some studies may be possible in people who are exposed to cyanide accidentally—for example, from acute smoke inhalation or chronic cassava poisoning.

1759 Cobinamide, Sodium Tetrathionate, and Sodium Nitrite/Sodium Thiosulfate for Treatment of Cyanide Poisoning

G. Boss. *University of California San Diego, San Diego, CA.* Sponsor: D. Jett

Cobinamide is a stable vitamin B12 (hydroxocobalamin) analog that is far more potent than cobalamin as a cyanide antidote. It can therefore be administered by intramuscular injection and could be given via an autoinjector. It also is an effective antidote against hydrogen sulfide and several other toxic chemicals, making it a potentially very useful drug. We have shown that intramuscularly injected cobinamide is highly effective in mouse, rabbit, and pig models of cyanide poisoning, rescuing >80% of animals from lethal doses of cyanide. Sodium tetrathionate, like sodium thiosulfate, is a sulfur donor that neutralizes cyanide by converting it to thiocyanate. Compared with thiosulfate, which neutralizes a stoichiometric amount of cyanide, tetrathionate neutralizes two moles of cyanide, thus reducing the amount needed to treat cyanide poisoning. Like cobinamide, intramuscular sodium tetrathionate rescues >80% of animals in the aforementioned mouse, rabbit, and pig models of cyanide poisoning. Sodium nitrite and sodium thiosulfate are currently approved for treatment of cyanide poisoning via intravenous injection. We have shown that sufficient amounts of the two drugs can be given by intramuscular injection to rescue animals in the mouse, rabbit, and pig models of cyanide poisoning.

1760 DTMS: A New Nonintravenous Candidate Cyanide Medical Countermeasure

G. Rockwood. *US Army Medical Research Institute of Chemical Defense, Edgewood, MD.* Sponsor: D. Jett

In industrialized countries, cyanide exposure most commonly occurs through inhalation of combustion smoke, but dangerous exposure also can occur through ingestion, dermal contact, and/or gaseous fume inhalation. While the current US FDA-approved cyanide countermeasures Cyanokit (hydroxocobalamin) and Nithiodote (sodium nitrite and sodium thiosulfate) are demonstrably efficacious against cyanide poisoning, both require intravenous (IV) administration and are consequently unsuitable for use in treating multiple cyanide victims, particularly at or near exposure sites a distance away from a hospital. In fact, hospital resources may be strained by an emergency need for multiple IV line preparation and implementation. Dimethyl trisulfide (DMTS) is a sulfur-based molecule present in garlic and onions and is currently listed by the US FDA as GRAS (Generally Recognized as Safe) for use as a flavor additive. DMTS, as an intramuscular (IM) anti-cyanide sulfur donor, is safe and efficacious in rodent models of cyanide injection, ingestion, and inhalation. In lethal rabbit and swine models of cyanide poisoning, DMTS significantly enhances survival. Recent improvements in DMTS formulation have yielded rodent pharmacokinetic results showing peak blood levels in as little as two to 10 minutes, and peak brain levels in 10 minutes, both post-IM injection. The potential role of DMTS as a viable cyanide medical countermeasure candidate will be discussed in the context of next-generation countermeasures that are practical, efficacious, safe, easy to administer, and cost-effective.

1761 Scaling Up: Phenotypic Screens and Testing of Novel Cyanide Countermeasures

C. Macrae[1], and R. Peterson[2]. [1]*Harvard University, Boston, MA; and* [2]*University of Utah, Salt Lake City, UT.* Sponsor: D. Jett

Several cyanide countermeasures already exist and are in clinical use, but their utility is limited by challenges related to mechanism of action and mode of delivery. Novel countermeasures remain a high priority, but validated targets are few. We have used a phenotype-driven screening approach to discover new compound classes that can counteract the effects of cyanide. Foremost among these are the multivalent cyanide scavenger hexachloroplatinate (HCP) and the metabolic modulator glyoxylate. A formulation of HCP has been developed that reduces its toxicity while enhancing its scavenging of cyanide. This HCP formulation has been shown to be efficacious in zebrafish, mouse, rabbit, and pig models of cyanide toxicity. Glyoxylate also has been proven efficacious as a cyanide countermeasure in zebrafish, mice, and rabbits. It exhibits low toxicity and a broad therapeutic window. Mechanistic studies have demonstrated that cyanohydrin formation represents only part of glyoxylate's rescue mechanism and that enzymatic transformation by lactate dehydrogenase is essential for glyoxylate's function. Importantly, both HCP and glyoxylate can be delivered by intramuscular injection. These discoveries pave the way for development of cyanide countermeasures that are fundamentally different from existing countermeasures and have the potential to transform our ability to respond to cyanide exposures.

1762 New Cobalt Complexes and NO Donors

J. Peterson. *University of Pittsburgh, Pittsburgh, PA.* Sponsor: D. Jett

While several cobalt complexes have been shown to exhibit cyanide-scavenging properties, only (hydroxo)cobalamin has, so far, been US FDA labeled for use in ameliorating the harmful effects of cyanide toxicity in patients without causing life-threatening side effects. Despite its apparent safety and clinical success, cobalamin is expensive and requires the slow (~15-minute) intravenous administration of large volumes. Finding less expensive and more rapidly effective cyanide antidotes would clearly be beneficial. Recently, we have shown a cobalt complex of the Schiff-base macrocycle 2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1] heptadeca-1(17)2,11,13,15-pentaene (CoN4[11.3.1]) to be a viable option as a cyanide antidote. CoN4[11.3.1] is a low molecular weight compound that binds two cyanide anions (cobalamin binds only one cyanide under physiological conditions) and is synthesized in a single step with affordable starting materials. The reaction kinetics of cyanide association and the stability of the dicyano adduct are demonstrated to be suitable for sequestering cyanide *in vivo* and elimination of the toxicant in the urine. In a sublethal mouse model, CoN4[11.3.1] is shown to be more effective at ameliorating cyanide toxicity when compared with cobalamin, its precursor cobinamide, or a cobalt-containing porphyrin. Significantly, no long-term neuromuscular sequelae are evident in these mice more than a week later as assessed by the ability of the animals to remain on a rotating cylinder (RotaRod testing). Due to its relatively low molecular mass (~four times smaller than cobalamin), CoN4[11.3.1] can be expected to exhibit greater molar solubility than other larger cobalt-containing cyanide antidotes, leading to enhanced ameliorative capability of CoN4[11.3.1] through faster delivery of effective doses. Ongoing work is investigating the use of CoN4[11.3.1] in combination with a nitric oxide (NO) donor to reactivate cytochrome c oxidase, with further improved antidotal capability anticipated.

1763 Electronic Cigarettes and Cardiovascular Toxicity: New Friend or Old Foe?

M. Olfert. *West Virginia University School of Medicine, Morgantown, WV.*

Popularity of and interest in electronic cigarettes (e-cigs) have grown rapidly and in large part due to marketing e-cigs as "harm-free" or "reduced-harm" products compared with cigarettes. These claims are based largely on observations that e-cigs have fewer toxicants than cigarettes and some evidence the e-cigs induce lower lung inflammatory responses compared with traditional cigarettes. Such a narrowly defined perspective toward health fails to consider that use of e-cigs (also known as vaping) is likely to have effects on other organ systems, too. For example, the cardiovascular system is well known to have significant comorbidities associated with inhalation of xenobiotic particulate matter (e.g., air pollution, smoking). Given that e-cigs have only been around for little more than a decade, robust studies evaluating and reporting the effects of vaping on cardiovascular function are only now beginning to emerge. Despite the current marketing dogma, the emerging evidence paints a bleak picture toward the overall benefits versus risk from e-cigs and opens many intriguing questions, such as (1) What component(s) in, or produced from, the e-liquid may be responsible for inducing cardiopulmonary and vascular dysfunction? (2) Does secondhand exposure induce the same health concerns? (3) Does the type of device (JUUL versus other devices), and/or temperature settings (wattage), change its harm potential? and (4) Are the long-term effects on vascular function the same across different vascular beds? This Symposium brings together a group of established investigators that have specific expertise with vaping and aerosol research who have begun to address these questions. Specifically the presentations will bring together data from *in vitro* cell and *in vivo* animal studies—that provide harbinger toward long-term cardiovascular health in humans—as well as direct studies on humans examining health and cardiovascular function. Collectively the talks are designed to provide the audience a broad, but in depth, journey on current knowledge and potential mechanisms underpinning cardiovascular toxicity from vaping. We anticipate that this Symposium can provide a framework for stimulating both public and scientific discussion on the relative benefits versus harm conferred by inhalation of novel tobacco products to overall health. Given the current interest and need for establishing public policy and regulation toward novel tobacco products, and particularly e-cigs, the speakers will be asked to discuss relevant available data regarding the potential positive and negative outcomes being observed that may complement or assist regulatory evaluations. The panel discussion also will specifically ask the speakers (and invite the audience) to participate in a discussion on the potential of the findings presented during the Symposium toward issues for regulatory concern.

1764 Electronic Cigarettes: Vapors' and Flavors' Effects on Cells

P. Talbot. *University of California Riverside, Riverside, CA.*

Human embryonic stem cells (hESC), mouse neural stem cells (mNSC), and respiratory epithelial cells are used as model systems to investigate how new tobacco products affect human health and determine the effects of environmental toxicants on early mammalian development. Our recent work has focused on electronic cigarettes and thirdhand smoke. Electronic cigarettes deliver nicotine without burning tobacco and are often thought to be less harmful than conventional cigarettes, although relatively little is known about their actual effects on human health. Our studies focus on the flavor chemicals used in electronic cigarettes and metals that appear in aerosols produced upon heating. Flavor chemicals are often present in e-liquids at high concentrations that exceed those normally used in consumer products, and metals enter aerosols from both the liquid and components in the atomizing units. Upon heating, flavor chemicals and solvents (propylene glycol and glycerin) can produce reaction products that may themselves be harmful. *In vitro* studies with 2D and 3D cultured cells have shown that e-liquids and aerosols vary in their cytotoxicity, that many electronic cigarette products contain concentrations of flavor chemicals that are cytotoxic in cell-based assays, and that there is significant variability in toxicity across the broad spectrum of electronic cigarette products currently available. The components in electronic cigarette aerosols that may impact the cardiovascular system and their possible effects will be discussed.

1765 Adverse Cardiovascular Effects of Aerosols from Leaf Vaporizers and E-cigarettes

M. Springer. *University of California San Francisco, San Francisco, CA.* Sponsor: M. Olfert

It is well known that exposure to tobacco smoke and other sources of particulate air pollution lead to adverse cardiovascular consequences, including endothelial dysfunction. We have shown that both mainstream and secondhand smoke from marijuana also impair endothelial function in rats. Based on this knowledge, a variety of ostensibly reduced-harm devices have become increasingly popular. Tobacco "heat-not-burn" products and marijuana leaf vaporizers, which heat plant material to subcombustion temperatures, are thought of as less harmful than their combustible counterparts. Liquid-based e-cigarettes that deliver nicotine or THC, including JUUL, are also considered to be reduced-harm products. While presumably less harmful than smoke, the aerosols produced by these products are increasingly recognized as harmful to cardiovascular, pulmonary, and other physiological systems. We showed that endothelial function measured by arterial flow-mediated dilation (a reflection of nitric oxide [NO]-dependent endothelial function) is rapidly impaired in rats by secondhand smoke from cigarettes, little cigars, and marijuana, and by mainstream smoke/aerosols from tobacco and marijuana, heat-not-burn tobacco products (IQOS), marijuana leaf vaporizers, and liquid-based e-cigarettes, including JUUL. Neither nicotine nor THC is required for this impairment. Exposure of cultured endothelial cells to serum from chronic e-cigarette users results in lowered production of NO, as has previously been reported for serum from cigarette smokers, reflecting a common mechanism of endothelial dysfunction on the cellular level. These

results are particularly important with regard to marijuana smoke and leaf- and liquid-based vaporizers because these are all viewed by some as being less harmful than tobacco smoke. Findings such as these can play an important role in informing the US FDA policies toward regulating these products. Similarly, as marijuana is being legalized in progressively more US states and other countries, awareness that it is not just a drug but another source of secondhand smoke is important for the public and political debates over how to regulate public use. Our findings, in agreement with results from other research groups using different approaches, indicate at this point that the best way to avoid adverse cardiovascular effects of inhalation is to breathe clean air.

1766 Cardiovascular Toxicity of Electronic Cigarettes: Role of Aldehydes

D. J. Conklin. *University of Louisville, Louisville, KY.*

Electronic cigarettes have emerged as a popular electronic nicotine delivery system (ENDS). Carbonyls including short-chain, toxic aldehydes have been detected in e-cig-derived aerosols at levels found in tobacco smoke. Similarly, the cardiovascular toxicity of acute and chronic ENDS exposures in humans is understudied, but recent results are providing warning signs regarding sensitivity of the cardiovascular system to ENDS. To address this gap in our knowledge, we assessed the relationship between aldehyde exposure and cardiovascular outcomes including acute hemodynamic changes and chronic changes in atherosclerosis lesion burden in mice. AIdehydes were generated in aerosols derived from tobacco products, from propylene glycol (PG):vegetable glycerin (VG) mixtures and from commercial e-liquids with flavorants as measured using a state-of-the-art carbonyl trap and mass spectrometry. We tracked mainstream cigarette smoke (MCS) and e-cigarette aerosol exposures in mice via urinary metabolites of aldehydes using ULPC-MS/MS and GC-MS as needed. Aldehyde levels, independent of abundance (saturated: formaldehyde, acetaldehyde >> unsaturated: acrolein, crotonaldehyde), were dependent on the PG:VG ratio and the presence of flavorants. Similarly, the metabolites of three aldehydes were increased in urine after both e-cigarette aerosol and MCS exposures, but the major crotonaldehyde metabolite (3-hydroxy-1-methylpropylmercapturic acid, HPMA) was increased only after MCS exposure. Exposure to menthol-flavored e-cigarette aerosol increased both levels of urinary 3-HPMA and the sum of nicotine exposure (nicotine,cotinine,trans-3′-hydroxycotinine) relative to exposure to tobacco e-cig. Acute exposure to e-cig aerosol (with or without nicotine) led to rapid onset and reversal of bradycardia with proarrhythmic changes in the ECG. Several of these changes were phenocopied with acrolein exposure, suggesting a role of aldehydes in these changes. Chronic exposures of apoE-null mice to MCS, e-cig with nicotine, acrolein, or crotonaldehyde for 12 weeks led to significantly increased atherosclerosis in all groups when compared with filtered air control group. These results demonstrate that long-term use of tobacco products containing nicotine and/or aldehydes induce cardiovascular harm in mice and, thus, growing concern for cardiovascular disease risk in humans who use ENDS.

1767 Toxicological Effects of Vaping on Central and Peripheral Vascular Function: Insights from Intravital and *In Vivo* Studies in Animals

M. Olfert. *West Virginia University School of Medicine, Morgantown, WV.*

Novel tobacco products, such as electronic cigarettes (e-cigs) and heat-not-burn (HNB) tobacco devices, are being marketed as harm-reduction or reduced-risk smoking products. In fact, very little is known about the safety and potential for harm of these devices to overall health and in particular on vascular health. Using murine and rodent exposure paradigms, we have tested the hypothesis that e-cigs and HNB products will show evidence of reduced cardiovascular toxicity. This presentation will provide evidence from acute exposures on peripheral vascular function obtained using intravital microscopy with exposure to e-cigs and HNB. We also examine the effects resulting from individual components of the e-liquid (i.e., nicotine, VG alone, PG alone). The effects of chronic exposure (i.e., 8-month) on *in vivo* assessments of cardiac function and aortic vessel reactivity are presented, in combination with *ex vivo* myography studies on central vessel (aorta) reactivity responses. Lastly, this presentation will show emerging data on central and peripheral vasculature function in offspring of rats that have been maternally exposed (during pregnancy) to e-cig vapor with or without nicotine. This presentation will contain data from both published and unpublished studies, with the expectation that it can help to lay a foundation for understanding the mechanisms underpinning the cardiovascular toxicity associated with the evolution of these novel tobacco products.

1768 Electronic Cigarettes versus Nicotine Replacement Therapies: Cardiovascular Implications

C. Delles. *University of Glasgow, Glasgow, United Kingdom.* Sponsor: M. Olfert

E-cigarettes have overtaken nicotine replacement therapies to become the preferred nicotine replacement product to support quit attempts. Despite the potential harm reduction associated with e-cigarettes, their role as a smoking cessation aide remains controversial and has raised global debate. Research into the cardiovascular and other adverse effects of e-cigarettes is challenging for a number of reasons. First, there is a paucity of well-designed human physiology studies into biomarkers and intermediate cardiovascular phenotypes, and robust long-term outcome data are not (yet) available to truly estimate the risk of e-cigarettes at the population level. Second, although preclinical studies can be useful, the choice of model depends on the expected adverse effects, and no model is able to truly reflect all aspects of human cardiovascular pathophysiology. Third, while e-liquids only contain a limited number of apparently safe ingredients, the process of heating and vaporizing produces hundreds or thousands of intermediate compounds with unknown safety profile. To complicate matters, the effects of nicotine (e.g., on the renin-angiotensin-aldosterone and sympathetic nervous systems) have to be separated from effects of other components of the inhaled product. Fourth, the technology advances quickly whereas research and legislation lag behind, making it difficult to compare studies or draw conclusions from data on "old" technology to "new" technology. These challenges translate into uncertainty among health care professionals regarding the risks and benefits of e-cigarettes, although there are good reasons to believe that e-cigarettes have a more favorable risk profile than tobacco cigarettes. This presentation also will highlight examples of research into the cardiovascular effects of e-cigarettes from a clinician's perspective, focusing on (1) the association between e-cigarettes and global cardiovascular risk, (2) immediate and intermediate effects of e-cigarettes on vascular function and structure, and (3) cardiac effects of e-cigarettes, including risk of myocardial infarction. Regulatory constraints surrounding research into smoking in smoke-free clinical research environments will be discussed.

1769 Environmental Exposure and Health Effects of Organophosphate Esters

D. Volz. *University of California Riverside, Riverside, CA.*

Organophosphate esters (OPEs) are high-production volume chemicals used worldwide within a number of products, including but not limited to electrical and electronic equipment, furniture, building materials, and vehicle parts. OPEs are additive flame retardants (FRs) and plasticizers that have the potential to migrate from end-use products into the ambient environment. As such, OPEs are ubiquitous in the environment and elevated within various sources of human exposure. As the extent and magnitude of exposure are likely associated with human population densities, this has raised concerns about the potential health risk to humans and the environment within densely populated regions around the world. However, OPE-specific toxicity data and epidemiological studies are relatively limited, although numerous studies have demonstrated that chronic human exposure is common within countries relying on OPE-containing materials. Therefore, the objective of this timely and globally relevant Symposium is to bring together several leading investigators that are actively conducting research on OPE exposure and effects within a range of environmental media and model systems. After a brief introduction of the Symposium and each of the speakers, the first speaker will provide an overview of OPE-specific uses, market trends, and regulatory challenges; the second and third speakers will focus on OPE exposure within the natural and built environment; and the last two speakers will focus on OPE effects on early and later life stages of vertebrate development.

1770 OPE-Specific Uses, Market Trends, and Regulatory Challenges

J. Tenney. *Israeli Chemicals LTD Industrial Products Group, Tarrytown, NY.* Sponsor: D. Volz

OPEs are an important class of FRs. The market growth for these chemistries is estimated to be around 5% annually. A major driver of this demand is fire safety standards in electronics, building and construction, and transportation applications. Market demand also is stimulated by continued interest in alternatives to brominated or chlorinated FRs. This presentation will review FR market trends, OPE functionality as an FR choice, major OPE uses/innovation, and regulatory challenges impacting FR selections.

1771 OPEs in the Biotic Exposome and the Influence of Environmental Processes on Fate and Biological Effects

R. J. Letcher. *Environment and Climate Change Canada, Ottawa, ON, Canada.*

OPEs have a variety of uses, including as FRs, plasticizers, pesticides, and additives to engine oils. Halogenated OPEs have been used as FRs as far back as the 1960s, and the phase-out of the polybrominated diphenyl ether (PBDE) FRs resulted in the substantial increase in the last 15 years in the production volumes and usage of an increasing array of OPEs. OPEs have a wide range of physical and chemical properties, ranging from very polar to very hydrophobic. Long-range transport of some OPEs has been shown based on concentrations reported in air (particle) samples. Atmospheric OPEs can be found at concentrations that are orders of magnitude higher than those of FRs such as PBDEs. Relative to abiotic environments, there is a growing body of evidence reporting much lower and mainly nondetectable OPE concentrations in biota. Degradation and transformation processes (including metabolism) and protein binding are proving to be major factors affecting OPE behavior in the environment and biological effects *in vitro* and *in vivo* in exposed organisms. This presentation will examine the current knowledge of OPE environmental and physiological behaviors in biota and biological effect outcomes, and the influence of chemical properties and degradation processes, which are relevant in comparisons with humans.

1772 Human Exposure to OPEs: Exposure Pathways, Predictors, and Health Concerns

H. M. Stapleton. *Duke University, Durham, NC.*

OPEs are commonly used in building materials and consumer products as FRs and plasticizers. The application of these chemical additives in textiles and polymers in particular has grown considerably over the last two decades, particularly following the phase-out of brominated FRs, and raising concerns about exposure among the general population and risk for adverse health outcomes. Our research group has conducted several studies among various populations (pregnant women, children, and adults) to characterize exposure among the general public and evaluate variables influencing exposure. This presentation will highlight our findings on exposure levels, demographic variables related to exposure, and associations with health outcomes. Specifically, we will discuss (1) the various OPEs identified in building materials (e.g., insulation) and consumer products (e.g., furniture, electronics); (2) measurement of exposure biomarkers in the general population, highlighting the higher concentrations observed in infants relative with adults, and in pregnant women compared with nonpregnant women; (3) the influence of age, BMI, and parity on exposure measurements; and (4) associations between exposure to OPEs during pregnancy and birth outcomes. This research is critical to conducting thorough and sound risk assessments for human health.

1773 Leveraging Zebrafish to Unravel Mechanisms of OPE-Induced Developmental Toxicity

D. C. Volz. *University of California Riverside, Riverside, CA.*

A better understanding of the potential effects of prenatal exposure of OPEs is needed. Based on our research over the last decade, this talk will highlight how our studies within zebrafish have enabled us to begin uncovering the mechanism of developmental toxicity for two high-production volume OPEs—tris(1,3-dichloro-2-propyl) phosphate (TDCIPP, a chlorinated phosphate ester) and triphenyl phosphate (TPHP, an unsubstituted aryl phosphate ester)—that exhibit unique modes of action during embryogenesis. Specifically, our more recent data show that (1) TDCIPP exposure at the beginning of cleavage (0.75 hours post-fertilization, hpf) results in delayed epiboly progression and disruption of dorsoventral patterning, phenotypes that may be due to alterations in epigenetic reprogramming within the first few hours of development; and (2) TPHP exposure from 5-72 hpf interferes with cardiac looping and heart chamber development, a phenotype that may be due to direct or indirect disruption of retinoic acid signaling pathways during organogenesis. Overall, our findings and ongoing studies within zebrafish (1) provide a foundation for understanding the impacts of TDCIPP and TPHP exposure on embryonic development, and (2) help formulate mechanistic-based hypotheses for studies within human cell-based models and prenatal developmental toxicity studies within mammalian models.

1774 Sex Differences in the Placental Accumulation and Activity of OPEs: A Novel Route of Neurodevelopmental Toxicity

H. B. Patisaul. *North Carolina State University, Raleigh, NC.*

The placenta is a critical source of hormones and neurotransmitters for the developing brain, particularly in early embryonic and fetal development. Thus, it may be an underappreciated but important target to consider in the context of developmental neurotoxicity. The commercial mixture Firemaster 550 contains two brominated compounds, bis (2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH, also known as BEH-TEBP) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB, also known as EH-TBB), and two OPEs including triphenyl phosphate (TPHP, also known as TPP) and a mixture of isopropylated triarylphosphate isomers (ITPs). Prior work by us and others has shown that gestational exposure can produce sex-specific effects on anxiety and exploratory-related behaviors. However, the mechanism of action remains unclear. Using Wistar rats, we showed that TBB, TBPH, and TPHP dose-dependently accumulate in placenta, with higher levels of TBPH and TPHP in male-associated placentas. Using a mixture of hypothesis generating (RNA sequencing and untargeted metabolomics) and targeted (qRT-PCR and targeted neurotransmitter analysis) approaches to examine fetal Wistar rat brain and placenta, we found that FM 550 likely impacts neurodevelopment by multiple mechanisms, including disruption of neurotransmitter, endocrine, and inflammatory pathways. Ongoing work with an OPE mixture is continuing to reveal how toxicity at the level of the placenta can alter neurodevelopment.

1775 Quantitative and Translational Systems Toxicology: Improving Drug Safety from Preclinical to Clinical Outcomes

J. Stevens. *Universiteit Leiden, Leiden, Netherlands.*

Before market approval, all new medicines require extensive preclinical and clinical safety testing. Molecules deemed safe in preclinical studies are generally safe during clinical trials, yet safety signals and adverse events do occur in human subjects. Catastrophic outcomes, although rare, do occur, and recent high-profile failures illustrate the potential for significant morbidity and mortality. Thus, predicting when preclinical results indicate increased clinical risk remains challenging and highlights the need for more quantitative and translational safety approaches. Systems approaches can be useful in estimating when biological response in one species is preserved in another. Various modeling approaches, including modular and network models, have been used to determine conservation of biology across scales of evolution. These systems models provide opportunities to place safety prediction on a more quantitative basis, especially if they are combined with PBPK models to link exposure to pathogenesis in a multi-scale quantitative systems toxicology (QST) framework. Multi-scale QST models incorporate data across vast scales of complexity, from cells to intact organisms, and may include data from the genome to the proteome and metabolome. For example, transcriptomic data can inform global metabolic models and provide measures of cellular stress response that are linked to metabolism and disposition of a novel drug candidate within an adverse outcome pathway that spans molecular responses to pathogenesis *in vivo*. Presentations in this Symposium will tackle various aspects of QST modeling. The session opens by addressing challenges in merging systems models with traditional PBPK models into complex multi-scale and multi-dimensional QST models. After this introduction, the presenters will focus on case studies illustrating the potential to improve translation of nonclinical to clinical safety for common organ targets, including liver, kidney, gut, and heart. The speakers will summarize progress in the field, illustrate new concepts that are nearing application, and highlight the opportunities and challenges in achieving more quantitative estimates of human safety.

1776 Challenges and Opportunities for Multi-Scale and Multi-Dimensional Models

C. Fisher. *Certara UK Limited, Sheffield, United Kingdom.*

Fundamental to any QST model is an understanding of the linkages between exposure and critical biological response parameters underlying safety and pharmacology. Physiologically based pharmacokinetic (PBPK) models integrate population-specific data (systems parameters) and compound-specific parameters to predict the absorption, distribution, metabolism, and elimination of compounds in the body, and subsequently enable the translation of these processes across populations and preclinical species. Such models represent a branch of quantitative systems pharmacology (QSP) and can act as a hub for the integration of models encompassing biological resolution ranging from the physiology to tissue and transcript levels. PBPK simulation of tissue/tissue substructure exposure profiles can provide the operating con-

centrations for tissue/cellular-level QSP models capturing mechanisms underlying cellular division, differentiation, death, repair, and core metabolism. These models can be further informed based on data from 'omics datasets (e.g., transcriptomics, proteomics, and metabolomics), and integrated models can range from the qualitative to the quantitative in their encoding and output. The ultimate aim is a multi-scale modeling framework spanning levels of biological organization and mechanistic resolution integrating multiple data sources. This will enable an iterative workflow of hypothesis formulation, model-based prediction, testing, and model refinement operating in parallel to, and simultaneously informing, the discovery and development pipeline, from target identification through to market. Using experiences and expertise from the IMI-TransQST project consortium, this talk will introduce the potential of such an approach and summarize the current state of the art and remaining challenges ahead.

1777 Modeling Stress Responses across Scales of Complexity: From High Content Imaging in Cells to Modeling Co-expression Networks

B. van de Water. *Universiteit Leiden, Leiden, Netherlands.*

Drug safety testing often begins in cell-based models before progressing to clinical and nonclinical studies. Drug-induced liver injury (DILI) and drug-induced kidney injury (DIKI), subsequent to cellular injury, remain an important concern in drug development and an area of high need for QST modeling and translational safety. Cellular stress response pathways are critical for adaptation to cell injury and can signal progression to adverse cellular and tissue outcomes. This presentation focuses on modeling drug-induced cellular stress response pathways across different scales of complexity. First, high content single live cell confocal imaging of GFP-tagged protein captures the dynamics of stress-responsive transcription factors (e.g., Nrf2, Xbp1, p53, and NF-kB) and corresponding downstream target proteins, allowing detailed quantification of cellular stress-responsive and inflammatory pathways after drug treatments. The detailed temporal- and concentration-dependent dynamics of stress pathways are used to develop computational models of the stress responses linking drug treatment to liver and kidney toxicity. Secondly, *in vitro* stress responses are linked to *in vivo* stress response pathways by building gene co-expression networks for both liver and kidney. Activation levels of the various stress gene networks by the cellular stress-responsive transcription factors noted here are assessed systematically and quantitatively for both DILI and DIKI compounds. We have further assessed the association of the various cellular stress-response network perturbations with adverse pathological outcomes in liver and kidney. Ultimately, mapping cellular stress-response network activation and the preservation of networks across species will enable quantitative translational risk assessment as new drugs enter clinical trials.

1778 Modeling Cardiovascular Safety Using a Quantitative Systems Pharmacology Approach

D. Leishman. *Lilly Research Laboratories, Indianapolis, IN.*

Cardiac function and frank injury to the myocardium itself are among the primary causes of attrition in both clinical and nonclinical studies. Computational models of cardiac electrophysiology and hemodynamics have been around since the '60s and '70s, respectively. This presentation will describe the use of these mature models and their further refinement to address critical questions relevant to changes in cardiac function during drug development. The presentation also will illustrate how these can be combined with PBPK models to move beyond pharmacological properties and in to patient-dependent intrinsic and extrinsic factors. The format of the presentation will follow an introductory overview of the models with case study vignettes addressing key questions drug development teams face. The case studies will involve examples related to cardiac electrophysiology, cardiac contractility, blood pressure, and heart rate. The talk will illustrate how the use of PBPK and functional models are integrated in asking the questions, Will my molecule delay cardiac repolarization (QTc)? and Will my molecule be proarrhythmic? In addition, examples will demonstrate how hemodynamic modeling can be used to address the question, What is the mechanism for the blood pressure change I observe with my molecule? Finally, the talk will illustrate a modeling approach to addressing whether or not a compound will be associated with cardiac hypertrophy. Overall examples will address cardiac electrophysiology, cardiovascular function, and structural toxicity.

1779 Multi-Scale Modeling Approaches to Describe Drug-Induced Gut Toxicity

C. Pin. *AstraZeneca, Cambridge, United Kingdom.* Sponsor: J. Stevens

The gut not only is a primary route of drug absorption but also is a well-known target for anti-proliferative compounds and can be dose limiting in clinical studies if the relationship between exposure and disruption of gut homeostasis is poorly understood. The dynamic nature of gut biology, including rapid cell turnover and unique immune functions, makes it a complex tissue with unique challenges. Multi-scale models enable the prediction of toxicity and recovery at multiple spatial and temporal scales. They are instrumental to quantify how target organs respond to toxicological challenges as a whole and at each structural level. This presentation will discuss modeling approaches able to describe the propagation of drug toxicity across scales in the gastrointestinal (GI) epithelium and how these approaches may enable quantitative predictions of GI clinical adverse effects based on the compound mechanism of action. As an example, data and models generated within the TransQST project wil be introduced to demonstrate how multiscale quantitative approaches can integrate the spatiotemporal cell dynamics of the GI epithelium with the dynamics of relevant proteins that regulate the cell cycle. The use of a computational model to simulate the impact of transcriptional changes, driven by clinically relevant PBPK models of cytotoxic drugs, on the cell cycle and the subsequent cascade of events spanning from single cell stress and/or apoptosis to the perturbation of cell migration across the crypt-villus axis with altered cell turnover and ultimately loss of epithelial integrity will be demonstrated. In summary, the application of QST multi-scale models to connect the molecular level with the clinic and predict emergent properties with impact on the discovery of new pharmacological properties and prevention of on-target GI toxicity will be demonstrated.

1780 Human-Relevant Carcinogenicity Testing: Tools for Cancer Assessment in 2020 and Beyond

N. Kleinstreuer. *NIEHS/NICEATM, Research Triangle Park, NC.*

For decades, risk assessors have relied on the rodent cancer bioassay to collect apical endpoints that are used to identify potential human carcinogens. The rat and mouse bioassays are currently required by most regulatory authorities for carcinogenicity assessment of industrial and agro-chemicals, food additives, pharmaceuticals, and environmental pollutants. However, five decades of cancer research have raised concerns about the utility of the rodent cancer bioassays to accurately identify potential human carcinogens. Timely discussions have reached a tipping point due to known limitations of the bioassay, including that it is expensive and time-consuming, uses hundreds of animals per test, and has questionable human relevance. As a result, experts are working to develop novel, human-relevant tools and approaches to assess potential carcinogenic effects. Steps are now being taken to modernize carcinogenicity testing through the use of mechanistic approaches that reduce testing on animals and may provide more human health-protective information. In this session, cross-sector experts will deliver presentations and participate in a panel discussion, providing insight into current challenges and opportunities in designing human-relevant chemical carcinogenicity assessment strategies. A representative from the National Toxicology Program will discuss their ongoing Health Effects Innovation Initiative that is working toward modernizing their carcinogenicity testing program. An investigator from the US Environmental Protection Agency will give a presentation highlighting the use of gene expression endpoints in short-term animal tests to predict liver tumorigens. Methods developers will discuss *in silico* expert-driven systems to develop testable biological hypotheses, small model organisms providing mechanistic insights, and complex organotypic model tumor systems that *rec*Apitulate human cancer. These presentations from a diverse panel of experts will provide a timely and thought-provoking discussion on the opportunities to modernize cancer assessment and will be of interest to a wide range of stakeholders, including regulators, cancer toxicologists, systems modelers, and industry scientists.

1781 Modernizing the NTP Carcinogenicity Testing Program

W. Casey. *NIEHS/NTP, Research Triangle Park, NC.*

Since the early 1980s, the US National Toxicology Program has served as a national and world resource for conducting the two-year rodent bioassay to assess the (human) carcinogenic potential of industrial and agricultural chemicals, food additives, environmental pollutants, and other exposures of public health concern. However, the acknowledged limitations of the rodent bioassay include high cost, extensive time to conduct and report, and limited

translational relevance. In building on its history of innovation, excellence, and impact, the National Toxicology Program is now leading the development of a new framework for assessing human cancer risk from environmental exposures. This talk will discuss the approaches being developed to better understand and interrogate the initiation/promotion and progression processes of human carcinogenesis as well as the etiology of a set of human cancers potentially associated with environmental exposures. The rationale for selecting these specific cancer targets and progress made on developing the associated adverse outcome pathways will be discussed. Other topics covered will include a description of ongoing efforts to develop a suite of *in vivo*, *in vitro*, and *in silico* approaches to screen and prioritize substances, utilization of longitudinal human cancer data, and opportunities for future engagement with stakeholders.

W 1782 Toward Patient-Specific Organotypic Models of Cancer

D. Beebe. *University of Wisconsin–Madison, Madison, WI.* Sponsor: N. Kleinstreuer

A critical piece of cancer assessment for chemical safety is the development of human-relevant models that can be used to more reliably predict human clinical outcomes and can in turn be applied to understanding environmental chemical contributions to carcinogenesis. Micro-scale organotypic tumor models hold promise because they use smaller numbers of cells, thus enabling the construction of patient-specific models from biopsy samples. In addition, the model can incorporate multiple cell types (e.g., cancer, stromal, and vascular) in more relevant context (e.g., ductal structures). We have developed organotypic tumor models across a range of patients in both breast and kidney cancer. These models exhibit more *in vivo*-like characteristics (e.g., reduced proliferation rates as compared with 2D culture) as well as increased sensitivity to xenobiotics. The development of these human organotypic tumor models will aid in mechanistic understanding of human cancer outcomes as well as provide enhanced testing approaches for the assessment of potential human carcinogens.

W 1783 Development of an Adverse Outcome Pathway Network to Support an Integrated Approach to Testing and Assessment of Carcinogenic Risk

S. Stalford. *Lhasa Limited, Leeds, United Kingdom.* Sponsor: N. Kleinstreuer

The prediction of human carcinogenicity and related toxicity endpoints has always been a principal area of concern during chemical safety assessments. Historically, the two-year rodent bioassay has been used as the ultimate method of assessing human carcinogenic risk. However, this type of assay is expensive and time-consuming and is not always accurate in extrapolating to humans. More recently, alternative methods have been developed to predict carcinogenic risk, including both *in vitro* and *in silico* techniques. However, while there is some guidance on how to integrate specific pieces of evidence in well-defined use cases, a unified approach to combination has yet to emerge, and new techniques are developed all the time. One approach to creating a framework in which this evidence could be combined in different ways would be to utilize the concept of adverse outcome pathways (AOPs). Using information already contained within Derek Nexus (DX), an expert rule-based SAR system with a well-developed knowledge base for carcinogenicity-related endpoints, and literature review, a carcinogenicity AOP network has been developed and expanded upon using evidence from the literature to create a network containing 27 molecular initiating events that link to the adverse outcome of carcinogenicity via more than 400 pathways. This network can be used in combination with Derek Nexus alerts as a rudimentary profiling tool for carcinogenicity mode of action prediction. Presenting more detailed information on potential pathways by making predictions in this way allows for integration of existing and emerging *in vitro* and *in vivo* test results at the protein level with predictions to support an overall conclusion on carcinogenic potential. This approach to knowledge presentation also allows for easier interpretation of the evidence available relating to a given prediction.

W 1784 Toward Replacing the Two-Year Bioassay with Short-Term Assays: Gene Expression Thresholds Can Predict Rat Liver Tumorigens

C. Corton. *US EPA, Research Triangle Park, NC.*

Traditional data sources for cancer hazard assessment are resource intensive, retrospective, and not feasible for the vast majority of environmental chemicals. Incorporation of quantitative genomic data from short-term rodent studies may adequately define protective thresholds for potential tumorigens as a bridge to move from current testing to greater reliance on *in vitro* assays. We hypothesized that gene expression biomarkers that measure the activation of the major molecular initiating events (MIEs) in rodent liver cancer adverse outcome pathways exhibit chemical-independent thresholds beyond which cancer occurs and the thresholds could be used to predict liver cancer. The hypothesis was tested by defining thresholds of gene expression biomarkers of liver cancer MIEs using training sets from the 77 and 86 chemicals in the TG-GATES and DrugMatrix datasets, respectively, and testing them in a number of contexts. The biomarkers tested, consisting of 7-113 genes, included those that predict genotoxicity, cytotoxicity, and activation of AhR, CAR, ER, or PPARa. Thresholds were calculated as the maximum values derived from exposures that do not lead to liver cancer. In all cases, clear threshold values could be identified that were consistent across training and test sets. Thresholds derived from the TG-GATES study were not very predictive of liver tumorigens in the DrugMatrix study (77%-81%). In contrast, thresholds derived from the DrugMatrix study were predictive in the TG-GATES study (84%-100%). The DrugMatrix-derived thresholds were most predictive when applied to test sets of 7d and 14d treatments (100% and 99%, respectively). In addition, thresholds derived from just 12 genes (two/biomarker) exhibited high predictive accuracy (up to 94%). These findings support the idea that early genomic changes can be used to establish threshold estimates or "molecular tipping points" that are predictive of later-life outcomes. *This abstract does not reflect US EPA policy.*

W 1785 Using Preclinical Models to Understand Metastasis

K. Tanner. *National Cancer Institute, Bethesda, MD.* Sponsor: N. Kleinstreuer

Much of our knowledge on tumor progression and metastasis has been obtained using murine models, where dissecting the role of the physical microenvironment in regulating organotropism remains technically challenging. The zebrafish is rapidly becoming a model for studying tumor behavior at different stages of the metastatic cascade.[1,2] Moreover, organs that are frequent sites of metastasis in human patients are well conserved between mammals and zebrafish. Our previous work has quantified tissue mechanics and architecture of diverse organs to assess direct comparisons between zebrafish and mammalian systems. We recently developed a zebrafish model of human tumor metastasis and human macrophage migration and demonstrated that it recapitulates patterns of metastasis of human tumors in mouse models. We discovered that human breast cancer cells that home to specific organs in mice also show nonrandom extravasation in an organ-specific manner within zebrafish. Specifically, cells that home to the murine brain also home to the zebrafish brain, whereas cells that home to the murine bone marrow also home to the embryonic bone marrow niche in larval fish. Extravasation was mediated by ß1 integrin, where knockdown of ß1 integrin reduced extravasation for both clones in the CVP but did not affect extravasation of brain-tropic clones in the brain. In contrast, silencing unconventional myosin 1b redirected brain tropic clones from the brain to the CVP. *References: 1. Follain, G., et al. "Hemodynamic Forces Tune the Arrest, Adhesion, and Extravasation of Circulating Tumor Cells."* Dev Cell *45, 33-52 e12, doi:10.1016/j.devcel.2018.02.015 (2018). 2. White, R., Rose, K., and Zon, L. "Zebrafish Cancer: The State of the Art and the Path Forward."* Nat Rev Cancer *13, 624-636, doi:10.1038/nrc3589 (2013).*

W 1786 Using Human Genetics to Aid in Safety Assessment of Therapeutics

J. Yuan. *Amgen Inc., Cambridge, MA.*

Preclinical safety evaluation of pharmaceuticals relies mainly on studies in animal models. While the use of animals has made significant contributions to safety assessment, there remains uncertainty in the extrapolation of risk from animals to humans. This is particularly true for novel therapeutics that do not cross-react to animal orthologs and lack pharmacological activity in species used for toxicology studies. Therefore, development of human-centric approaches to predict drug safety in humans is essential. Research in human genetics has discovered hundreds of gene variants associated with complex and rare disease. Both studies of Mendelian syndromes and genome-wide associ-

ation studies (GWAS) have resulted in the discovery of new therapeutic targets and have provided therapeutic support for existing drugs. Retrospective analyses of clinical studies have demonstrated that drugs whose targets are validated by human genetics are more likely to reach approval. While the application of human genetics has been discussed primarily as a method to discover and validate the efficacy of novel targets, there is increasing appreciation for the use of genetic data to predict safety liabilities. Human genetic variants can represent a model for lifelong modulation of a therapeutic target and can be used to inform the potential for on-target toxicity as well as the effects of drug off-target interactions. In addition, human genetics may help to identify genetic risk factors contributing to rare adverse drug reactions. The overall objective of this Workshop is to showcase the use and potential of human genetics to inform safety signals of therapeutics. The session will cover the following topics: (1) an overview of human genetic association studies including GWAS and PheWAS, with the aim of providing a basic understanding of the methodologies and applications to the drug development process; (2) case examples that illustrate the applications of human genetics in target validation and to inform on- and off-target safety—the impact on drug safety evaluation and clinical utility will be discussed; (3) the utility of human genetics in identifying genetic risk factors contributing to adverse drug reactions from a large volume of health records as well as knowledge accumulated from drug development; and (4) discussion on the challenges of and future perspectives on translating human genetic information to predict drug effects in preclinical and clinical development. This session will be of interest to toxicologists engaged in preclinical and clinical safety evaluation during drug development and to human geneticists interested in preclinical applications and clinical translation.

W 1787 Genetics for Target Validation and Case Studies

D. Diogo. *Merck Research Laboratories, Boston, MA.* Sponsor: J. Yuan

Human genetics serves as an "experiment of nature" to help inform drug discovery. Naturally occurring mutations ("perturbation") may affect the function of a gene ("target") and lead to reproducible effects on clinically relevant phenotypes ("clinical indication" and/or "adverse drug event" [ADEs]). These genetic instruments thus mimic the effect of therapeutic modulation of the target and can help to predict dose-response curves at early stages of target validation. Human genetic data linking the target gene to the clinical indication (predicting drug efficacy) have been demonstrated to increase the success rate of drugs in clinical trials. Examples of approved or failed drugs also illustrate the potential of human genetics to retrospectively predict ADEs, both on-target and off-target. In this section, we will discuss how Mendelian genetics (disease-causing mutations implicated in Mendelian syndromes) and population genetics (rare-to-common genetic variants found in the general population and associated with disease predisposition) can be leveraged to predict efficacy and safety. We will introduce the concepts of genome-wide association study (GWAS), phenome-wide association study (PheWAS), and Mendelian Randomization, and will illustrate, through selected retrospective examples, how these methods can be applied to inform efficacy, identify potential alternative indication, and predict potential on-target or off-target ADEs. We also will discuss the potential of rapidly growing biobank initiatives, linking genome-wide genetic data with extensive health information in large cohorts of participants, to accelerate efficacy and safety predictions. We will conclude by discussing some challenges and limitations of human genetics to inform drug discovery and development.

W 1788 Using Genetics to Select Safer Targets and Drugs

P. Nioi. *Alnylam Pharmaceuticals, Cambridge, MA.* Sponsor: J. Yuan

In drug development, early-stage genetic validation often focuses more on efficacy than safety. Here, we present the results of systematic analyses that support two ways in which human genetics can be used, even preclinically, to improve safety: anticipating target-mediated side effects and prioritizing off-target screening of drug candidates. In a retrospective analysis, we found a correlation between the organ systems affected by genetic variation in drug targets and the organ systems in which side effects were observed during clinical trials. This result suggests that human genetic data can be used to help predict target-driven drug safety issues and should be integrated into safety assessments. A key consideration for drug safety is not only the biology of the intended target, but also the effects on secondary "off-targets." Based on an analysis of marketed drugs, we recommend that genetics be used to guide counter screening during drug development. We anticipate that integrating genetics into on- and off-target safety assessment will help to reduce safety-related drug failures.

W 1789 Observational Data for Pharmacogenomics Discovery

N. P. Tatonetti. *Columbia University, New York, NY.* Sponsor: J. Yuan

Observation is the starting point of discovery. Based on observations, scientists form hypotheses that are then tested. In the information, trillions of observations are being made and recorded every day—from online social interactions to the emergency room visit. With so much data available, generating hypotheses using a single scientist's mind is no longer sufficient. Data mining is about training algorithms to recognize patterns in enormous sets of data and automatically identify new hypotheses. This presentation will discuss how we use data mining algorithms to characterize drug response in ancestrally diverse patient populations (NYC EHR data) and use large repositories of genetic data (both local and the UK Biobank) to identify genetic risk factors of adverse drug reactions. The presentation will demonstrate that statistical machine learning can produce patient phenotypes even when primary diagnosis data are unavailable. This allows us to use noisy databases, like the electronic health records, for genetics and pharmacogenomics studies.

W 1790 The Role of Genetics and Genomics in Predicting Drug-Induced Liver Injury

W. Tong. *US FDA/NCTR, Jefferson, AR.*

Drug-induced liver injury (DILI) is a serious safety concern, with >1,000 drugs being reported to possess the potential to cause liver injury. However, most DILI occurs at low frequency (idiosyncratic) and some are genetically driven. Despite the vigorous and extensive safety testing during the drug development process, DILI remains an enigma. We have been developing the Liver Toxicity Knowledge Base (LTKB) for an enhanced assessment of DILI with emerging methodologies, including genomics and genetic methodologies. In addition to drugs' innate properties, mechanistically relevant cellular endpoints from *in vitro* assays, and histopathology findings, LTKB utilizes the pharmacogenomics information and patients' phenotypic responses to drug treatment to understand the underlying genetic mechanisms for DILI. The goal of LTKB is to develop a content-rich resource to improve understanding of liver toxicity and ultimately for the US Food and Drug Administration to utilize and reference when liver toxicity issues arise during various stages of the regulatory review process. This presentation will provide an overview of the LTKB, with a specific emphasis on applications of the genetic and genomic data to assess and predict DILI.

W 1791 Integrating Genetic Evidence into Drug Safety Research: From Target Discovery through Pharmacovigilance

M. Nelson. *Deerfield, New York, NY.* Sponsor: J. Yuan

Our session presenters will share the latest advances in our understanding of the capabilities and limitations to use genetic insights to reduce risks of developing new therapies that have safety hazards through understanding who is at increased risk of experiencing adverse events and using genetic information to reduce them. In this synopsis, we will summarize the state-of-the-science to reaffirm the opportunities to impact drug discovery, development, and post-marketing pharmacovigilance, as well as to highlight the gaps in understanding and practice for these insights to benefit patients.

PL 1792 Does Variability in Ontogenetic Maturation Trajectories Explain the Variability in Drug Pharmacokinetics for Preterm and Term Birth Neonates?

J. Fisher[1], D. Mehta[1], and J. Troutman[2]. [1]*US FDA/NCTR, Jefferson, AR; and* [2]*Procter & Gamble, Mason, OH.*

In 2016 the FDA reported a lack of PBPK model performance in describing the pharmacokinetics of drugs in pediatrics. A deep-dive into model parameter assumptions for a neonate PBPK model was accomplished, which included creating longitudinally based growth curves and data-derived estimates for model parameter variability. A preterm and term neonate model was constructed to predict plasma concentrations for a combination drug Piperacillin (PIP) and Tazobactam (TAZ) in 31 neonates. To evaluate the model performance, model predicted and observed plasma concentrations of PIP and TAZ were compared without any adjustment of model parameters. Armed with predicted 90 percentile distribution intervals for PIP and TAZ plasma concentrations, the PBPK model failed to predict the observed plasma PIP

levels in only 12% of the neonates for PIP and 17% for TAZ. For the remaining preterm and term neonates nearly 50% of the neonates were deemed acceptable and the remaining neonates were categorized as mixed, with 2 or more plasma concentrations outside 90 percentile distribution intervals for each drug. The model performance outcomes were very promising given that the PBPK model was not calibrated by adjusting model parameters. The variability in the drug plasma concentrations was greater than the predicted variability based on distribution assumptions for maturation of body weight, organ volumes, blood flows, cardiac output, plasma protein binding, GFR, and tubular secretion. Future efforts to improve performance includes the characterization of neonatal illness, refined descriptions for model parameters, and expanding the number of drugs for simulation purposes.

1793 Enhancing Stem Cell-Based Toxicity Assays by Engineering the Culture Niche in a High-Throughput Assay-Agnostic Manner

N. A. Geisse. *NanoSurface Biomedical Inc., Seattle, WA.* Sponsor: N. Geisse, American Society for Pharmacology and Experimental Therapeutics

Human induced pluripotent stem cells (hiPSC) hold great promise for detecting adverse events *in vitro*, but there are limits to their ability to accurately model human physiology. For example, hiPSC-Cardiomyocytes (CMs) misclassify several compounds of known cardiotoxicity. Cell maturity is a major contributor to this; most hiPSC-CMs express fetal phenotypes, leading to inaccurate or difficult-to-translate results. Extracellular matrix (ECM) engineering improves both structural and functional phenotypes, but is challenging to perform on industry-standard assays and instrumentation platforms. Engineering increasingly intricate artificial culture environments often drives decisions to trade off biological complexity with experimental throughput. Here, we developed biomimetic patterns that mimic the shape and structure of the ECM on transparent polymers which direct focal adhesion assembly mechanisms in individual iPSC-CMs and drive higher-ordered tissue architecture. These 2D cardiac syncytia are formed in industry-standard microplate formats compatible with light microscopy. The method is also extended to micro-electrode arrays (MEA) in a manner that maintains both the quality of MEA recordings and baseline electrophysiological properties. iPSC-CMs grown this way show adult-like structural and biochemical phenotypes like protein isoform expression, myofibril alignment, and sarcomere dimensions. We show that biomimetic cues enhance the electrophysiological response of cardiomyocytes to various drugs of known effect. Since ECM structuring recapitulates the polarized nature of gap junctions, we used the connexin blocker carbenoxolone for validation. Our data show that structured tissues enable differential conduction block measurements across longitudinal and transverse directions and that patterned tissues have increased sensitivity to the drug (IC50s of 14.71 vs. 398.1 nM, respectively) and compare favorably to clinically relevant unbound concentrations. Our approach is instrument- and assay agnostic, effective on a variety of cell types and lines (including neuronal cells), and compatible with high-throughput approaches. We conclude that bioengineering techniques can enhance the predictivity and maturity of iPSC-CMs in culture.

1794 Optimization of UGT Inhibition Assays: Evaluation of Substrate and Inhibitor Selectivities

J. Reinen, M. Smit, and M. Wenker. *Charles River, 's-Hertogenbosch, Netherlands.* Sponsor: H. Emmen

Glucuronidation is the major Phase II drug metabolizing pathway which is catalyzed by UDP-glucuronosyltransferases (UGTs). A significant number of drugs are known to be metabolized by UGTs, either directly or following Phase I metabolism. Inhibition of UGTs by xenobiotics may alter the pharmacokinetics of drugs that are eliminated primarily via glucuronidation. It is therefore very important to evaluate the potential UGT inhibition for new chemical entities (NCEs) during drug development. In human liver, seven major UGT enzymes (1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15) are present and assays to determine the inhibition of these enzymes have been developed. In the current study, we have evaluated the use of different substrates and inhibitors in order to implement assays to study the inhibition of the seven major UGT isoforms. Based on literature research, β-estradiol (E2) and SN-38 (both 1A1), CDCA (1A3), trifluoperazine (1A4), serotonine (1A6), propofol (1A9), zidovudine (2B7), oxazepam (2B15) and the general UGT substrates 4-methylumbelliferone and 7-hydroxycoumarine were selected as substrates. Analytical methods (UPLC-PDA-MS) were developed and implemented for each substrate and the corresponding glucuronide metabolite and incubations with human liver microsomes were performed to evaluate the selectivities of the selected substrates. Specificity for the intended UGT was confirmed for SN-38, CDCA, trifluoperazine, serotonine, propofol, zidovudine and oxazepam (S-oxazepam-glucuronide formation only) whereas E2 was found to be metabolized by multiple UGTs. Subsequently, protein- and time-dependent experiments were performed to optimize the reaction conditions for the UGT-specific substrates to ensure that reactions followed first-order kinetics and that substrate depletion was below 30%. The optimized reaction conditions were used to evaluate the inhibition potential of a selection of known inhibitors (erlotinib, troglitazone, hecogenin, niflumic acid, diclofenac and ketoconazole) for each of the seven UGTs. The current study gives an overview of assays that can be used to study UGT inhibition and therefore is of added value for risk evaluation of UGT-mediated drug-drug interactions (DDIs).

1795 New Statistical Modeling Strategy to Dissect the Effects of Drug Properties and Host Factors on Toxicity Using Postmarket Surveillance Data

D. Wang, K. K. Khadka, and M. Chen. *US FDA/NCTR, Jefferson, AR.*

Due to the well-known limitations for current animal testing based approaches to drug toxicity, there is heightened interest in both high throughput *in vitro* assays and epidemiological data. Epidemiological data sets are especially important for dissecting the effects of drug properties, host factors, and their interactions in effecting drug toxicity. But comprehensive data sets fit for this purpose are very difficult to obtain. Postmarket surveillance databases such as the FDA Adverse Event Reporting System (FAERS) database represent a rich but underutilized data source for data mining and model development in this area. Current applications using FAERS data commonly focus on detecting drug-adverse event (AE) combinations with unusually high relative reporting rates while using FAERS data for model development and validation regarding drug properties or host factors is relatively rare. This is partly due to the lack of well-developed statistical methodology. In this paper, we developed a novel statistical approach that incorporates drug properties and host factors in the modeling of relative reporting rates using large postmarket surveillance databases like FAERS. It is a natural extension of current data mining approaches for detecting drug-AE combinations with unusually high relative reporting rates like multi-item Gamma Poisson Shrinker and likelihood ratio tests. By incorporating covariates in a Poisson regression framework, the effects of drug properties or host factors on toxicity can be estimated and tested. In one case study, we demonstrated that a predictor that we constructed in a previous study with AOP networks and *in vitro* assays is very significantly associated with a drug's relative reporting rates ($p<10^{-6}$), thus collaborating the model using high throughput assays. We also demonstrated the proposed model can be used to identify drug-AE combinations with significant sex disparity in relative reporting frequency. With further development, this method has potential to enable extensive studies regarding the interplay between drug properties and host factors for drug toxicity using publicly available postmarket surveillance databases.

1796 Revisiting the hERG Safety Margin after 20 Years of Routine hERG Screening

D. Leishman, M. A. Abernathy, and E. B. Wang. *Eli Lilly and Company, Indianapolis, IN.* Sponsor: L. Buckley

It has been two decades since screening new molecules and potential clinical drug candidates against the hERG potassium channel became a routine part of safety pharmacology. The earliest heuristic for what was an adequate safety margin to separate molecules with a liability to cause the arrhythmia torsade de pointes (TdP) from those with no such liability emerged in 2002 and was determined to be 30-fold the therapeutic free plasma Cmax (Webster et al., 2002). In the intervening years nonclinical and clinical ICH guidances have been introduced and intense scrutiny has been applied to the QT interval of the electrocardiogram in animals and man. Has the 30-fold heuristic stood the test of time? The hERG margin between the IC_{50} value and the therapeutic unbound plasma concentrations were examined for 351 compounds. These margins were compared against the categories used by www.CredibleMeds.com to classify a drug's TdP risk. A subset of 320 of these drugs were compared against their US product labels with respect to black box warnings on QTc prolongation or TdP, warnings and precautions on QTc or TdP and QTc language in the clinical pharmacology section. Against the CredibleMeds Classification the modes of the distributions of margins for Known, Possible, Conditional Risk of TdP, and Not Listed (presumably no TdP liability) were 5, 17, 56 and 468, respectively. Against the US label language the modes of the distributions of margins for black boxes and warnings were 5 and 10, respectively. The margins associated with, positive QTc outcome, negative QTc outcome and no QTc language were 8, 355 and 52, respectively. The 30-fold heuristic would still appear to hold with respect to the US label language falling between the margins for negative QTc outcome or no QTc language and positive QTc outcome, warnings or black boxes. Similarly, the

30-fold mark separates drugs with a known or possible TdP risk from those where it is at best conditional and certainly from the 231 drugs not listed on www.CredibleMeds.com. The fact that the margins in each category form distributions is not surprising but a more consistent manner of assessing hERG potency and evaluating relevant exposures would likely reduce the spread in these distributions making margins even more useful as a decision-making data point.

1797 Assessing the Poly-Pharmacology of Kinase Inhibitors by Transcription Factor Activity Profiling

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Protein kinases are promising drug targets for multiple diseases. The challenge in developing therapeutic PK inhibitors (PKI) stems from the fact that most PKIs interact with multiple kinases and non-kinase targets (PKI polypharmacology). Existing evaluation approaches afford kinome-wide profiling of PKI activity but do not consider the biological effects of PKIs and their interactions with non-kinase targets. Here, we describe an effect-based methodology to biological evaluation of PKIs. Under this approach, we assess cell response by profiling the activity of multiple transcription factors (TFs), proteins connecting signaling pathways with regulated genes. Previously, we demonstrated that TF activity profiles (TFAPs) provided robust quantitative signatures of compounds, permitting straightforward identification of perturbed biological processes[1]. Using a multiplexed reporter assay[2] (the FACTORIAL™) in human hepatocytic HepG2 cells, we evaluated TFAP signatures for a panel of over 120 PKIs in a broad range of concentrations. (1) We found that inhibitors for the same kinase shared an identical TFAP signature, regardless of structural dissimilarities and MOA (ATPase vs. allosteric inhibitors); furthermore, kinase-dead cDNA mutants produced the same signature. Therefore, this invariant signature is a bona fide marker for inhibited kinase; (2) we found distinct invariant signatures for multiple kinases (Raf, Mek, Erk, mTOR, Akt, and Aurora); (3) the invariant signatures for kinases of the same vertical pathway (Raf/Mek/Erk; mTOR/Akt) had a high-degree similarity, suggesting a straightforward approach to mapping kinases within the signaling network; (4) the TFAP signatures of most PKI changed with concentration; by querying those TFAPs against the invariant signatures for inhibited kinases and landmark perturbagens, we determined the concentration windows wherein the dominant effect of a PKI was an inhibition of a particular kinase or a perturbation of a non-kinase processes (e.g., mitochondrial malfunction, DNA damage, proteotoxicity). Our methodology is a powerful addition to existing techniques, illuminating biology that is invisible to target-based screening. The TFAP signatures provide quantitative metrics for assessing PKI polypharmacology by revealing the dominant effects on kinases and non-kinase targets. This approach also suggests a new methodology to map the human kinases in the context of vertical pathways and to deorphanize the dark kinases. *References: 1. Medvedev, A.* et al. Sci. Adv. *4, eaar4666 (2018). 2. Romanov, S.* et al. Nat. Methods *5, 253-60 (2008).*

1798 Investigative Safety Strategies to Reduce Risk of Drug-Induced Cholestasis and Hepatobiliary Toxicity in Drug Discovery and Development at Bristol-Myers Squibb Company

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Drug induced liver injury (DILI) is a major cause of drug attrition in the pharmaceutical industry and altered bile acid homeostasis has been hypothesized to cause cholestasis and liver injury. BMS has withdrawn drug candidates from development due to clinical evidence of hepatobiliary toxicity. One such candidate was the LPA_1 receptor antagonist BMS-986020. Data with structurally distinct LPA_1 antagonists indicated that the toxicity from BMS-986020 was compound- rather than LPA_1-mechanism-based. Retrospective studies with BMS-986020 to assess the mechanism for hepatobiliary toxicity demonstrated inhibition of key hepatic canalicular and sinusoidal bile acid transporters (i.e., BSEP, MRP3, MRP4) by BMS-986020 at relevant clinical drug exposures. BMS-986020 also inhibited taurocholate efflux from human hepatocytes into bile and blood, and inhibited the efflux of phospholipids from human hepatocytes into bile mediated by MDR3. In agreement, BMS-986020 increased plasma bile acid levels in rats and humans. Moreover, BMS-986020 altered mitochondrial function in human hepatocytes and cholangiocytes, and caused hepatobiliary toxicity in monkeys. Based on these data, data for other BMS compounds, and literature reports, an investigative preclinical testing strategy was developed for BMS compounds that inhibit BSEP, the primary hepatic bile acid efflux transporter. This testing strategy progresses sequentially through tiers from early discovery to early development. Tier 0 considers *in vitro* high throughput inhibition data for BSEP. Tier 1 compares projected clinical plasma drug concentrations to the BSEP inhibition data, and Tier 2 incorporates data for risk enhancers (noncompetitive BSEP inhibition, inhibition of additional bile acid efflux transporters and MDR3, hepatic drug accumulation, and increased bile acids *in vivo*) and risk reducers (inhibition of hepatic bile acid uptake transporters NTCP and OATP). In Tier 3, additional DILI liabilities (altered mitochondrial function, oxidative stress, reactive metabolites) and *in vivo* nonclinical evidence of hepatobiliary toxicity are considered for an integrated DILI risk assessment. Taken together, this study presents the data to support an investigative strategy of drug-induced perturbations of bile acid homeostasis, cholestasis, and hepatotoxicity for preclinical and clinical programs.

1799 Understanding the Applicability of *In Silico* and *In Vitro* Safety Models to Improve the Risk Assessment of Drug Candidates

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Many *in silico* and *in vitro* safety models are used during lead optimization to identify safety-related liabilities. Most are validated using diverse sets of compounds in an attempt to understand a model's predictive potential. However, most of these models are not perfect in terms of their performance against the training set and it is essential to understand under what situations a model is or is not predictive. For example, through an assessment of an *in silico* model of *in vitro* 3T3 phototoxicity, the use of the HOMO-LUMO gap descriptor was shown to be most important factor towards the prediction of 3T3 activity. A deeper analysis, however, showed that this descriptor was only useful for describing the phototoxic potential of compounds without a fused-ring system and not those with fused-ring systems, highlighting a predictivity gap within the model. With respect to *in vitro* systems, we assessed the applicability of several cytotoxicity assays in terms of their ability to predict toxicity in preclinical *in vivo* safety studies. Cytotoxicity assays using HepG2 and HUVEC showed good performance for predicting toxicity findings below the total Cmax level of 10 uM (AUCs by ROC curve analysis were 0.679 and 0.696, respectively) but this was not the same for human primary hepatocytes (ROC AUC = 0.574). The *in vivo* toxicity profile of those compounds that were cytotoxic only towards primary hepatocytes was diverse and not restricted to the liver. This suggests that it is challenging to interpret the cytotoxic activity of a candidate drug in human primary hepatocytes. A further analysis, however, indicated that they may be useful for the determination of toxicity mechanisms, particularly with respect to metabolism. For example, a compound that was only active in human primary hepatocytes showed metabolite-related toxicity in the kidney, indicating that primary liver cells can identify the risk of metabolite-related toxicity in various organs, not only in liver. Several case studies within Takeda will be presented to show the importance of understanding the value of *in silico* and *in vitro* models through structural and mechanistic investigation of the training set. Knowledge learnt from these analyses cannot only identify predictive knowledge gaps, but also uncover toxicological knowledge that can be used to build more effective safety screening paradigms.

1800 De-Risking Biotherapeutic Lead Selection: How Specific Is Your Antibody?

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Poor specificity frequently derails drug discovery and over half of preclinical safety failures are caused by off-target binding. We developed the Membrane Proteome Array (MPA) platform to de-risk lead selection by testing biotherapeutics for specificity and off-target binding. This platform tests specificity across 6,000 human membrane proteins, each expressed in live cells. In contrast to commonly used Tissue Microarrays (TMAs), proteins in the MPA exist in their native conformations and are not altered by fixation. Binding interactions are assayed by high-throughput flow cytometry allowing for high sensitivity detection and rapid analysis. In the process of testing hundreds of antibodies, we found that up to 20% of antibodies exhibit detectable off-target binding. In many cases, off-target interactions occurred with unrelated proteins and could not be predicted by protein sequence homology. We used our high-resolution Shotgun Mutagenesis epitope mapping platform to elucidate these unintended interactions and better understand the nature of unpredicted off-target binding.

1801 Iodoacetic Acid Affects Estrous Cyclicity, Ovarian Gene Expression, and Hormone Levels in Mice

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The disinfection of drinking water was a major public health achievement of the 20th century. However, the reaction between disinfectants and organic matter in water generates water disinfection by-products (DBPs). Iodoacetic acid (IAA) is one DBP that has been shown to be an ovarian toxicant *in vitro*, but its effects on the ovaries *in vivo* are not well known. This study determined whether IAA exposure affects estrous cyclicity, ovarian expression of genes that regulate apoptosis, the cell cycle, steroidogenic factors, estrogen receptors, and the levels of reproductive hormones in mice. Adult CD-1 mice were dosed with IAA (0, 0.5, 10, 100, and 500 mg/L) in the drinking water for 35 days and estrous cyclicity was monitored for 14 days. After 35 days, ovaries were collected for analysis of expression of apoptosis regulators (*Bax, Bok, Aimf1, Bcl2* and *Bcl2l10*), cell cycle regulators (*Ccna2*, *Ccne1*, *Ccnb1*, *Ccnd2*, *Cdk4,* and *Cdkn1a*), steroidogenesis factors (*Star*, *Cyp11a1, Cyp17a1, Cyp19a1, Hsd17b1,* and *Hsd3b1*), and estrogen receptors (*Esr1* and *Esr2*). In addition, sera were collected to measure pregnenolone, androstenedione, testosterone, estradiol, inhibin-B, and follicle stimulating hormone (FSH) levels. IAA exposure decreased the time that the mice spent in proestrus compared to control. Further, IAA exposure decreased expression of the pro-apoptotic factor *Bok* (100 and 500 mg/L), the cell cycle regulator *Ccnd2* (500 mg/L), and borderline decreased expression of the anti-apoptotic factor *Bcl2l10* (10 mg/L), the pro-apoptotic factor *Aimf1* (0.5 mg/L), and the steroidogenic factor *Cyp19a1* (10 and 500 mg/L) compared to control. In contrast, IAA exposure increased expression of the pro-apoptotic factors *Bax* and *Aimf1* (500 mg/L), the anti-apoptotic factor *Bcl2l10* (500 mg/L), the cell cycle regulators *Ccna2*, *Ccnb1*, *Ccne1*, and *Cdk4* (500 mg/L), and the estrogen receptor *Esr1* (500 mg/L) compared to control. IAA exposure did not affect expression of *Star*, *Cyp11a1, Cyp17a1, Hsd17b1, Hsd3b1,* and *Esr2.* Further, IAA exposure decreased estradiol levels (500 mg/L), but did not alter pregnenolone, androstenedione, testosterone, inhibin-B, and FSH levels. Collectively, these data show that IAA exposure alters estrous cyclicity, ovarian gene expression, and estradiol levels in mice. *Supported by NIH R21 ES028963 and NIH T32 ES007326.*

1802 Earlier Puberty and Ovarian Germ Cell Depletion in Female Mice Exposed *In Utero* to Benzo[a]pyrene during Either Germ Cell Mitosis or Meiosis Onset Developmental Windows

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Polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (BaP), are products of incomplete combustion of organic materials. It has been shown that exposure to PAHs during ovarian development causes premature ovarian failure in rodents. Primordial germ cells (PGCs), embryonic precursors of oocytes, arise in the mouse yolk sac at 7.25 days postcoitum (dpc). PGCs proliferate before and after arriving at the gonadal ridge 10.5 dpc and begin entering meiosis at 13.5 dpc. Now oocytes, they arrest in the first meiotic prophase beginning at 17.5 dpc. This finite oocyte pool is the primary determinant of female fertility and reproductive lifespan. We have shown that *in utero* exposure of female mice to 2 or 10 mg/kg/day BaP during a dosing window that spans the mitotic and meiotic stages of PGC development results in depleted follicle numbers postnatally. We hypothesized that PGCs are more sensitive to BaP exposure during rapid proliferation, starting at 6.5 dpc prior to entry into meiosis at 13.5 dpc. We exposed timed-pregnant female mice to 0 or 2 mg/kg/day of BaP in oil, by oral pipetting, from 6.5-11.5 dpc or 12.5-17.5 dpc corresponding to germ cell mitosis and meiosis, respectively. Female offspring were examined daily for vaginal opening. Then vaginal cytology was conducted until first estrus to assess puberty onset. One female from each litter was euthanized for ovarian follicle counts on the morning of first estrus. The females exposed to BaP *in utero* displayed a 2-day earlier first estrus (P=0.005) compared to unexposed females, regardless of exposure window. Females exposed to BaP, regardless of timing, were sensitive to germ cell depletion. We observed statistically significant decreases in primordial, primary, and secondary follicle counts with BaP dose (P<0.02, 2-way ANOVA, N=5-8/oil, 9-11/BP), but no statistically significant effects of dosing window or the interaction between BaP dose and window (P>0.48). We observed little change in number of antral follicles for both dosing windows. These results support that germ cells are equally sensitive to BaP-induced cell death during mitosis and meiosis developmental windows and demonstrate that prenatal exposure to BaP depletes the finite ovarian reserve and hastens the onset of puberty. *This research was supported by NIH R01ES020454.*

1803 Prenatal Exposure to an Environmentally Relevant Phthalate Mixture Accelerates Reproductive Aging in Multiple Generations of Female Mice

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Phthalates are found ubiquitously in products including plastics and personal care products, resulting in daily human exposure. Phthalates are known endocrine disrupting chemicals that have been shown to negatively affect female reproduction. Previously, our laboratory found that prenatal exposure to a relevant phthalate mixture composed of 35% diethyl phthalate, 21% di(2-ethylhexyl) phthalate, 15% dibutyl phthalate, 15% diisononyl phthalate, 8% diisobutyl phthalate, and 5% benzylbutyl phthalate disrupted female reproduction in multiple generations of mice. However, it is not known if prenatal exposure to a mixture of phthalates affects reproductive aging and if these effects are accelerated compared to control. Reproductive aging in females is characterized by a decrease in the follicle pool, a lack of normal cyclicity, an increase in the occurrence of cystic ovaries, and a dysregulation of the hypothalamic-pituitary-gonadal axis, leading to decreased fertility. This dysregulation is defined as a decrease in sex steroid and peptide hormone levels and an increase in gonadotropin hormone levels. Thus, we tested the hypothesis that prenatal exposure to a mixture of phthalates accelerates reproductive aging in multiple generations of female mice. Pregnant CD-1 dams were orally dosed with control or a phthalate mixture (20 µg/kg/day-500 mg/kg/day) daily from gestational day 10 to birth. Adult F1 females born to these dams were used to generate the F2 generation by mating them with unexposed males and F2 females were used to generate the F3 generation. At 13 months, estrous cyclicity was monitored for 14 days and ovaries and sera were collected for analysis. Prenatal exposure to the phthalate mixture (200 µg/kg/day) decreased primordial follicle numbers and increased the occurrence of cystic ovaries in the F1 generation. The mixture (20 and 200 µg/kg/day, 500 mg/kg/day) increased time spent in metestrus/diestrus in the F3 generation. Further, the mixture decreased levels of progesterone (F2), testosterone (F1, F2), and inhibin B (F1), but increased levels of follicle-stimulating hormone (F1) and luteinizing hormone (F1, F3) in multiple generations of aging mice. These data suggest that prenatal exposure to a relevant phthalate mixture accelerates reproductive aging in a multi- and transgenerational manner in female mice. *Supported by NIH P01 ES022848 and EPA RD-83459301.*

1804 A Subcompartmental Analysis of Oxidative Stress in Ovarian Reproductive Aging

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Environmental toxicants cause tissue damage, in part through reactive oxygen species (ROS) formation. ROS also contributes to aging. Previous work from our group revealed an increase in ovarian fibrosis in old mice compared to young mice and this is associated with a significant decrease in total ovarian hyaluronan (p<0.05). Hyaluronan (HA) is a large (> 2 MDa) extracellular matrix (ECM) glycosaminoglycan that helps maintain tissue homeostasis and is abundantly expressed in the ovary. In extra-ovarian tissues, HA depolymerization to low molecular weight fragments (<250 kDa) by hyaluronidases and ROS can aid in tissue repair or, if persistent, can promote inflammation and fibrosis, both of which are hallmarks of ovarian aging. We hypothesized that ovarian ROS would be associated with HA loss in an age-specific and compartmental specific manner. To test this hypothesis, oxidative stress and antioxidant gene expression in whole ovaries or isolated ovarian stromal husks from reproductively young (6-12 weeks) and old (14-17 months) mice were analyzed using a pathway targeted QPCR array, followed by QPCR-based gene expression validation. 4-hydroxynonenal (4-HNE) immunohistochemistry was used to detect ROS-mediated ovarian macromolecule damage. HA was localized in ovaries from young and old mice followed by subcompartment quantification of HA-positive staining. The stromal compartment had a significant increase in the oxidant production-related genes Ncf1 and Ncf2 in old vs young mice (p<0.05). The antioxidant genes Txn1 and Hmox1 were significantly increased, while Gpx6 was decreased in old stroma vs young stroma (p<0.05). 4-HNE adducts were localized to the stroma, follicles, corpora lutea, and theca cells in both young and old mice; only theca-associated 4-HNE was greater in old mice (p<0.05). This was fascinating since we also observed a significant decrease (p<0.05) of HA in the theca layer in old mice vs young mice. Collectively, these data suggest that increased theca cell oxidant stress could contribute to HA loss in this ovarian subcompartment, but not in the non-theca stromal cell compartment, with advanced reproductive age. In conclusion, our findings indicate that ROS could be a potential mechanism by which HA is degraded in ovarian reproductive aging.

1805 Testing the Response of Pharmaceutical Compounds on Female Reproductive System Using Tissue Engineering and Microfluidic Culture Technologies

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The lack of optimal *in vitro* models for testing the safety of pharmaceutical compounds on female reproductive system makes the current gold standard dependent on *in vivo* animal studies. To aid in the rapid assessment of likely compound toxicity prior costly animal studies and to ultimately reduce or replace the use of animals in biomedical research, we have developed *in vitro* systems that are being tested against standard static well-based assessment modalities. To date, newly tested tissue engineered mimics and 3D microfluidic culture have produced more physiologically relevant environment *in vitro* and also allow the integration of multiple organ systems. Our next step is to determine if they provide an efficient and reliable pre-clinical tool for screening the response of pharmaceutical compounds on female reproductive system and functions. In this work, we used a combination of encapsulated *in vitro* follicle growth model (eIVFG) and a microfluidic culture model to screen for the impact of compounds under pharmaceutical development at AstraZeneca (AZ) that were stopped for safety concerns. Multilayered secondary ovarian follicles were isolated from 16-day-old CD-1 female mice and encapsulated within 0.5% alginate hydrogel. Follicles were then cultured using traditional static 3D culture, treated with various compounds at concentrations of 0, 0.1, 1.0, 10, or 100 μM for 24h, and then cultured for a total of 8 days. Using this approach, it was identified that while some compounds had no effect on follicle growth and survival, other compounds exhibited adverse impact at high-doses or in a dose-dependent manner. Additionally, ovarian explants were cultured using microphysiological systems (MPS), treated with concentrations of 0, 10 or 100 μM for 24h, and then cultured for 8 days. In microfluidic culture, compounds showed similar results to that in the static culture. These results illustrate the potential application of tissue engineering and microfluidic technologies in the investigation of reproductive safety of pharmaceutical compounds of interest.

1806 Integrated Genomic, Epigenomic, and Exposomic Analysis of Placentas from Preeclamptic Patients Identifies Links to Acetaminophen and Placental Cellular Damage Signaling

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Preeclampsia is a serious pregnancy disorder that results from the dysregulation of placental function that can be detrimental to both the mother and developing fetus. Preeclampsia impacts up to 8% of all pregnancies worldwide, yet the factors contributing to the development of this disease remain largely unknown. This study set out to test the hypothesis that understudied xenobiotic chemicals are capable of reaching the human placenta and disrupting critical pathways relevant to placental cell health and preeclampsia. Placentas from a previously established cohort of patients with normotensive (N=17) and preeclamptic (N=18) pregnancies were analyzed using non-targeted approaches based on high-resolution quadrupole time-of-flight mass spectrometry to identify unknown molecular features. Features were matched against multiple chemical inventories, including the Agilent MS/MS fragmentation database and the US Environmental Protection Agency computational toxicology database. Feature concentrations were statistically associated with preeclampsia disease status, as well as genomic and epigenomic signatures from the same placental samples. A total of 172 molecular features were identified with significantly increased levels in tissues from preeclamptic patients. One of the identified features showing the largest increase in concentration was acetaminophen. Concentrations of acetaminophen were found to be associated with altered expression of 26 genes and 57 miRNAs and altered methylation status of 18 genes relevant to preeclampsia. Acetaminophen-associated genes showed enrichment for cell death / damage signaling, which were further characterized through *in vitro* testing with immortalized trophoblasts. This research provides novel evidence towards characterizing the placental exposome and identified acetaminophen as one of the most significantly associated compounds related to preeclampsia. Mechanistic findings showed changes in signaling relevant to placental cell damage that may be relevant to preeclampsia etiology and support the need for further investigation. This study serves as an important foundation to further elucidate relationships between potential chemical exposures and placental health in pregnant women.

1807 Transcriptional Profiling of the Trichloroethylene Metabolite *S*-(1,2-Dichlorovinyl)-L-Cysteine Revealed Activation of the EIF2α/ATF4- Integrated Stress Response in the HTR-8/SVneo Trophoblast Cell Line

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Exposure to trichloroethylene (TCE) in pregnancy has been associated with reduced birth weight in humansand rats. Due to environmental contamination and continued usage, TCE exposure is an ongoing risk. The TCE metabolite *S*-(1,2-dichlorovinyl)-L-cysteine exhibits toxicity in a placental cell line. Thus far, investigations into the mechanisms of DCVC-induced cytotoxicity have been limited to narrowly-defined methods evaluating single molecular signaling pathways and biological responses. In the current study, genome-wide transcriptomics and gene set enrichment analyses were used to identify novel biological processes and molecular signaling pathways altered by human exposure-relevant DCVC concentrations in HTR-8/SVneo cells. Transcriptomics revealed 123 significantly up-regulated and 225 down-regulated genes with exposure to 20 μM DCVC for 12 h (FDR<0.05 and fold-change < -1.5 or >1.5). Gene set enrichment analyses demonstrated that the most substantially altered molecular signaling pathway was the EIF2α/ATF4 Integrated Stress Response (ISR), with the most abundantly altered Gene Ontology Biological Processes including amino acid transport, metabolism and biosynthesis, transcription and translation, and regulation of tissue development. Western blotting confirmed the involvement of the ISR. Our results provided insight into functional consequences in the cells. For example, decreased global protein synthesis was measured in agreement with ISR signaling. However, no changes in cell cycle progression or proliferation were detected, suggesting that a generally successful adaptive process occurred after 12 h of 20 μM DCVC treatment. This study provides further insights into the mechanism of DCVC-induced cytotoxicity by revealing the involvement of a specific stress signaling pathway.

1808 A Modified Parachute Assay for Accurate Assessment of Gap Junction Intercellular Communication in Placental Trophoblast Cells

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Gap junction intercellular communication (GJIC) can be assessed with a parachute assay, where fluorescent dye-loaded donor cells are seeded onto acceptors and dye diffuses to adjacent cells with active gap junctions. GJIC is expressed as the percent dye positive cells. Since cells remain in co-culture for up to 4 h, donor cells can attach, but the assay does not allow distinction between donors and acceptors, which is a major limitation. Updates to this method use DiI dye, but DiI can be effluxed by donors over time resulting in false positives. We aimed to develop a parachute assay with distinguishable populations and hypothesized that it will assess GJIC comparable to current methods such as the scrape load/dye transfer assay (SL/DT) and is more accurate than methods lacking donor detection. Since intercellular communication is required during placental development, we used the trophoblast cell line HTR8-SVneo as the acceptor and HTR8-SVneo expressing red-fluorescent protein (RFP) as the donor. The assay was optimized under positive-control conditions using PKA activator, CW008. We have previously shown that gestational exposure to bisphenol S (BPS) reduces binucleate trophoblast cell number in sheep by disrupting fusogenic cell signaling. Since GJIC is one of the first steps in cell fusion, we also tested if BPS alters trophoblast cell GJIC. Donors and acceptors were exposed for 24 h to DMSO (0.1%), CW008 (1 μM), BPS (200 ng/mL), 12-O-tetradecanoylphorbol 13-acetate (TPA; 10 nM; negative control), or combinations of the three. Donors loaded with calcein AM (10 μM) were then added at 10,000 cells/well onto acceptors. Cells remained in co-culture for 3 h then fixed, DAPI stained, and imaged. Transfer events were calculated as: Σcalcein positive cells minus ΣRFP positive cells over total cells; normalized to the control. BPS enhanced GJIC in HTR8-SVneo cells, like CW008. TPA significantly attenuated both chemical-induced enhanced GJIC. As predicted, exclusion of donors during quantification resulted prevented false positives. This is the first report of a parachute assay for measuring GJIC in placental cells. Like the SL/DT assay, this novel parachute method reliably

detects both enhanced and suppressed GJIC. The ease and accuracy of quantification over current methods make this new assay optimal for automation and a useful tool for *in vitro* toxicological placental testing. *Supported by NIEHS R01ES027863 to AVL. JG was supported by NICHD T32HD087166.*

1809 Per- and Polyfluoroalkyl Substances (PFAS) Inhibit Trophoblast Migration and the Expression of Inflammation-Related Genes

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Per- and polyfluoroalkyl substances (PFAS) are used as industrial surfactants and chemical coatings for household goods such as Teflon. Despite regulatory efforts to phase out historical PFAS perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), PFAS are detectable in drinking water throughout the United States. This is due to the stability of PFOS and PFOA and the continued use of replacement compounds, such as GenX. PFAS have also been detected in placenta and cord blood. Of concern for pregnant women, PFAS have been associated with low birthweight and increased preeclampsia risk in humans. Preeclampsia is a leading cause of maternal morbidity and mortality worldwide and is driven by insufficient trophoblast migration into the maternal endometrium, resulting in poor placental blood flow. PFAS alter migration of other cell types, but their impact on trophoblast migration is not understood. Allowing for mechanistic research, trophoblast migration can be assessed *in vitro*. We assessed the effects of PFAS on trophoblast gene expression and function *in vitro*. Trophoblast migration was assessed using a traditional *in vitro* scratch assay. The results showed that exposure to 1000 ng/mL PFOS, PFOA, and GenX was associated with decreased trophoblast migration over 24 h, with each PFAS having a similar effect. Treatment with PFOS, PFOA, and GenX also decreased trophoblast expression of chemokines (e.g. *CCL2, CCL7*), chemokine receptors (e.g. *CCR4, CCR7*), and inflammatory enzymes (e.g. *ALOX15, NOS2*) involved in migration. Prior reports have shown that PFAS alter migration and inflammation in other cell types through a peroxisome proliferator-activated receptor (PPAR)-dependent mechanism. We therefore tested the interaction of PFOS, PFOA, and GenX with PPARs using reporter cell lines. We determined that all 3 PFAS can both activate and inhibit (in the presence of a known ligand) one or more PPAR isoforms. Taken together, these data indicate that PFAS potentially decrease trophoblast migration. Moreover, by understanding the mechanisms involved, it may be possible to identify risk factors for and preventative measures against disease associated with failed trophoblast migration such as preeclampsia.

1810 Association of Maternal-Fetal Polybrominated Diphenyl Ether (PBDE) Levels with Biomarkers of Placental Development and Disease During Mid-Gestation

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While the mechanisms and periods of sensitivity remain undefined, *in utero* exposures to polybrominated diphenyl ethers (PBDEs) may be linked with placenta-mediated fetal and maternal health complications. To define the relationship between exposure to PBDEs and human placental development during mid-gestation, we performed a two-part analysis: 1) an evaluation of PBDE exposures in matched samples of maternal serum, placenta, and the fetal liver collected from healthy pregnant women undergoing elective termination procedures during mid-gestation (n=180; 2014-16); and 2) a semi-qualitative characterization of biomarkers of human placental development in relation to placental PBDE levels (n=62). All study protocols were approved by the University of California, San Francisco (UCSF) institutional review board prior to recruitment; written and verbal consent were obtained from each study participant at the time of enrollment. We used censored Kendall's tau correlation and maximum likelihood regression to compare PBDE levels between maternal-fetal tissues and examine their associations with biomarkers of placental development and disease. Specifically, we profiled placental cytotrophoblast (CTB) expression of integrin alpha 1 (ITGA1) and vascular endothelial-cadherin (CDH5) and metalloproteinase 1 (MMP1). In addition, we evaluated morphological features: leukocyte recruitment (basal plate), fibrinoid deposition (villous, basal plate), and CTB endovascular invasion. PBDEs were detected in all biomatrices. Prior to lipid adjustment, wet-weight levels of PBDE congeners were highest in the fetal liver compared to other compartments ($p<0.001$). In contrast, after lipid adjustment, PBDE levels were significantly higher in maternal serum compared to the fetal liver and placenta ($p<0.001$). We also observed significant associations between placental PBDE levels and endovascular CTB immunoreactivity of ITGA1 (inverse) and interstitial CTB immunoreactivity of CDH5 (positive), suggesting these markers of CTB invasion and development may be sensitive placental biomarkers of PBDE exposure. In summary, our work suggests that PBDEs are widely detected and differentially distributed in maternal-fetal compartments. Furthermore, we propose specific biomarkers of placental development as potential barometers of PBDE exposure during mid-gestation. This paradigm could be extended to other environmental chemicals and placental stage-specific antigens. *This work was supported by the US EPA (No. RD-83543301) and NIEHS (P01ES022841, R00ES023846).*

1811 Integrated Approaches to Testing and Assessment of Chemical Respiratory Sensitizers: A Weight-of-Evidence Assessment of Available Information to Derive a List of Reference Chemicals

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An Adverse Outcome Pathway leading to chemical respiratory sensitization has been outlined and, despite progress made in developing hazard identification approaches to assess chemicals for this endpoint as well as high regulatory need, there remains a lack of internationally harmonized approaches to identify chemical respiratory allergens. In order to assess potential *in vitro* and *in silico* approaches, a reference list of ~120 putative chemical respiratory sensitizers was generated based on structural alerts; a weight of evidence-based approach was undertaken to validate the reference list based on human clinical, *in vivo* non-human, and *in vitro* data. Well-defined criteria and a scoring matrix were used to curate the human data, taking into consideration the variability in tests performed by asthma clinics and laboratories alike, as well as uncertainties embedded in these analyses and reporting. To allow for a weight of evidence analysis, we are collecting all data but qualifying the data to reflect methodological quality. We are also making use of the Abstract Sifter literature review tool to identify additional potential respiratory sensitizers. Briefly, a set of PubMed MeSH terms describing adverse effects (AEs) for 92 known sensitizers was used to query a large database of chemicals and AEs, yielding over 7000 chemicals of potential interest. Work is ongoing to identify query terms which yield the most relevant papers. The resulting chemical list, which will be shared with regulatory agencies and the public, is an important step towards the assessment of potential test methods and the creation of internationally harmonized integrated approaches for the detection of chemical respiratory allergens. *Does not necessarily represent US EPA policy*.

1812 Maternal Arsenic Exposure Modulates Microglial Activation, Proliferation, and Phenotypic Transition in Developing Mouse Brain

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Arsenic (As) is a famous environmental toxicant and more than 200 million people worldwide are exposed to As. As is a known human carcinogen and its carcinogenic and systemic toxicity have been extensively studied. As can easily pass through blood-placenta and blood-brain barrier. As exposure alters cognitive function, particularly learning and memory during Childhood. However effects of As on microglia in developing brain have not been studied. Microglia originates from embryonic yolk sac in early embryonic days and colonizes neuroepithilium. In early postnatal development microglia has amoeboid morphology which is mitotically very active. During third postnatal week microglia attain the desired number, their proliferation reduces and transform into ramified morphology. In case of chronic maternal immune activation they cannot transformed into ramified shape. Runx1 is a key regulator of myeloid cell proliferation and differentiation in the postnatal brain. It inhibits microglia proliferation and promotes progression to ramified state. In this study we investigated the effect of arsenic on proliferation and phenotypic transition of microglia in developing brain upon maternal As expo-

sure. Female Balb/c mice were exposed 0.38 mg/kg sodium arsenite ($NaAsO_2$ from GD5 to PND22) through oral gavage. RTqPCR and western-blot were performed to detect expression of microglia phenotypic marker. Maternal As exposure induced the expression of amoeboid phenotype marker (CD86) in primary microglia as well as maternally-exposed developing brain. We have also found that maternal As exposure increased the level of secretary molecule like ROS and NO. As also altered the level of Chemokine, (RANTES, MIPα, MIP1β) and cytokines (IL1α, IL6 and TNF α) secretion determined by multiplex assay. RTqPCR, Western-blot and Immunoflorescence analysis showed that As induces the microglia activation (CD68), proliferation (CSF1R) and also induces the expression of RUNX1 in developing brain. These findings suggest that chronic maternal exposure to As altered microglial activation, proliferation and phenotypic transition that may lead to neurodevelopmental disorder.

1813 Comparative Effects of Exposure to Whole Cigarette Smoke and Tobacco Heated Vapor in Jurkat T Cells

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Cigarette smoking is a challenge for public health systems and government agencies, linked to high mortality rates worldwide. Cigarette toxicity is due to its combustion, which releases chemical compounds capable of damaging human physiology and leading to homeostasis imbalance. Among other toxic effects, conventional cigarette smoking is known to impair immune responses and the biological functions of cellular components, such as T lymphocytes, which are a major subset of immune cells responsible for mediating adaptive immunity. The effects of cigarette smoking on T cells are linked to either development or aggravation of diseases such as rheumatoid arthritis, ulcerative colitis and chronic obstructive pulmonary disease. Thus, non-combustible cigarettes ("heat-not-burn") have been developed aiming at reducing the toxic effects due to use of tobacco products, but data in scientific literature on their immunotoxic effects are still scarce. In the present work, we investigated the effects of exposure to cigarette smoke and tobacco heated vapor on T lymphocytes. For such, Jurkat cells were exposed to either cigarette smoke or heated tobacco vapor in an *in vitro* system composed of an air-liquid interface; in a exposure chamber coupled to peristaltic pumps, the cells were exposed to either smoke or vapor for 30 minutes in cycles of 2 second followed by 58 seconds of airflow. Cells were also co-stimulated with phorbol myristate acetate (PMA) to determine whether exposure to smoke or tobacco vapor could exacerbate the parameters evaluated in an inflammatory context. The viability of cells when exposed to cigarette smoke, stimulated or not with PMA, decreased significantly when compared to cells exposed to heated tobacco vapor or to airflow. Oxidative (reactive oxygen species and nitric oxide) and inflammatory (tumor necrosis factor alpha, interleukin 8, and interferon gamma) mediators were elevated in the cigarette-exposed groups compared to the heated tobacco vapor and airflow groups. Therefore, exposure to heated tobacco vapor caused less cytotoxic effects on Jurkat cells in comparison to conventional cigarette smoke.

1814 P,p'dde Exposure Modulates Nitric Oxide Production and Arginase Activity in Bone Marrow-Derived Macrophage from Mouse

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The Stockholm Agreement (2013) and the World Health Organization (WHO) allow using restricted for the DDT for disease vector control purposes. The Mexican population remains exposed to this compound and its metabolites, such as p,p'-DDE, the most persistent due to its lipophilicity. The DDT produces adverse effects on the endocrine, nervous, and immune systems, and it has been classified as a possible carcinogen by the IARC. Exposure to p,p'-DDE high levels are associated with systemic Lupus erythematosus, and in exposed individuals, elevated levels of IL-4 and Th2 cytokines, and decrement f Th1 cytokines have been observed in plasma, as well as increased lower respiratory tract infections. In macrophages of mouse cell lines, p,p'-DDE inhibits the ability to limit intracellular growth of *Mycobacterium microti* and yeast while increases the number of macrophages in adipose tissue. The objective of this study was to evaluate the effect of p,p'-DDE on macrophage's polarization to M1 and M2 phenotypes. The bone-marrow-derived macrophage (BMDM) was exposed to 0.125, 1.25, and 2.5 micrograms p,p'-DDE /mL and activated with cytokines: IFN-gamma /LPS (to M1) or IL-4/IL-13 (to M2). The p,p'-DDE exposure starts 12 h before the activation, and it remains until the end of the assays. Cell viability was assayed by MTT. Effects on the macrophages functionality were determined through a) the inflammatory phenotype was by the nitric oxide (NO) production, and b) by Arginase enzyme activity as a marker of the tissue repair. In macrophages activated with IFN-gamma /LPS, the p,p'-DDE does not affect the cell viability, significantly decreases the NO production, and does not affect the arginase activity at 72 h. In macrophages activated with 1L-4/IL-13, the p,p'-DDE does not affect the cell viability, neither the NO production, but significantly augments the arginase activity at 72 h. The effect of the p,p'DDE on other markers of macrophage M1 and M2 and the potential mechanisms by which the p,p'-DDE modulated this polarization of macrophages, is currently evaluated. *The study is funded by the Mexican Council for Science and Technology (Conacyt 32913).*

1815 Altered Immune Cell Migration Kinetics Contribute to Sex-Dependent Influenza Pathology in Arsenic-Exposed Adult Mice

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Inorganic arsenic (iAs) is a common drinking water contaminant and immunotoxicant associated with increased risk of infection. Respiratory infections like influenza A virus (IAV) remain among the top ten causes of death worldwide. We previously demonstrated increased IAV susceptibility in iAs-exposed mice, with females suffering greater morbidity and mortality than males. Females exposed to iAs exhibit a cytokine storm in the lungs at 3 days post challenge (DPC), but maintain higher lung viral titers by 9 DPC compared to control females and dose-matched males. However, it is still unknown how iAs exposure modulates immune cell infiltration and kinetics in both the mediastinal lymph nodes and lungs during infection and how this contributes to sex-specific pathology. We hypothesize that exposure to iAs skews the immune response in a sex-specific manner, contributing to the increased pathology and mortality observed in females. After 5-wks of 0 or 10 ppb iAs (sodium meta-arsenite) drinking water exposure, C57BL/6 mice were intranasally infected with a mildly lethal dose of mouse-adapted A/California/04/2009 [ma2009]. Results demonstrate that exposure to iAs causes significant changes in immune infiltration kinetics by 3 DPC. Cell counts reveal fewer cells in both the mediastinal lymph nodes (p=0.077) as well as the lungs (p<0.05) of males compared to control females. In both males and females exposed to 10 ppb iAs, CD4:CD8 T cell ratios in the lungs are non-significantly reduced at 3 DPC (p<0.10). Additionally, the median fluorescence intensity (MFI) of CD86 is significantly increased in the cells of *ex vivo* stimulated MHCII+ and MHCII- endothelial cells from the lungs of 10 ppb iAs-exposed females compared to control females and dose-matched males (p<0.05). This effect is also true of control females when compared to control males in the same cell populations (p<0.001). We are currently investigating how iAs exposure modulates B cell and innate immune cell populations at 3 DPC and how all of these populations continue to shift by 9 DPC. Our data suggest that sex-dependent immunomodulatory effects of iAs drive influenza infection pathology. These results help elucidate the increased respiratory pathology seen in epidemiological studies of individuals heavily exposed to drinking water iAs. *Funding: 5T32HL007534-37 (SEA).*

1816 The Interplay between Dietary Indole-3-carbinol, Th17 Cells, and the Microbiota in the Gut Influences T1D Development

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For nearly the past two decades, there has been a steady increase in the incidence of type 1 diabetes (T1D), an autoimmune disease that causes the destruction of insulin-producing beta cells. Susceptibility to T1D has been attributed to both genetic and environmental factors; however genetic predisposition alone cannot explain this rise. Understanding how environmental factors impact the immune response in individuals who are genetically susceptible to T1D can help in developing prevention methods. Primary interactions between the environment and the host immune system occur at mucosal sites, where both the diet and the microbiome have been identified as potential risk factors for T1D. One connection between the diet and the microbiome is the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor that modulates the expression of metabolizing enzymes (e.g. Cyp1a1) as well as immune genes. To better understand the extent by which a dietary AhR ligand can modify T1D development, we fed NOD mice a synthetic diet supplemented with 2000 ppm indole-3-carbinol (I3C; equivalent to 250 mg/kg/day). I3C is found in cruciferous vegetables such as broccoli, kale, and brussels sprouts. I3C highly activated AhR in the small intestine, as measured by Cyp1a1. AhR was also induced systemically, although to a much lower extent. Critically, after 5 weeks of supplementation, I3C increased

the degree of insulitis. To identify the mechanisms by which I3C may modulate the development of insulitis, we isolated the spleen, pancreatic draining lymph nodes, Peyer's patches, small intestine intraepithelial lymphocytes, and small intestine lamina propria lymphocytes and analyzed T-cell subsets (Th1, Th17, Foxp3+Tregs and Tr1). Dietary I3C did not alter these populations in the spleen, pancreatic draining lymph nodes, or intraepithelial lymphocytes. CD4+RORγt+Foxp3- (Th17 cells), however, were increased in both the lamina propria and Peyer's patches. Th17 cells significantly and positively correlated with the severity of insulitis in mice treated with dietary I3C. A follow-up study confirmed that the increase in CD4+RORγt+Foxp3- also corresponded with an increase in IL-17-producing T-cells in the lamina propria. Stool samples were taken prior to treatment and throughout the study for 16s rRNA sequencing. Data are currently being analyzed to predict networks connecting bacteria, intestinal Th17 cells, and insulitis.

1817 Propylene Glycol/Vegetable Glycerin and Menthol-Flavored E-cigarette Aerosol Induced Strain and Sex-Dependent Immune Toxicity in Mice

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Flavored e-cigarettes (e-cig) have become popular among teenagers in recent years. Due to flavor bans, the regulatory agencies are not sure about including menthol flavors among the other e-cig flavors that need to be banned. We have previously shown that menthol flavor induces reactive oxygen species and inflammation *in vitro* in human lung cells. In this study, we hypothesized that menthol-flavored e-cigarettes differentially affect the immune-inflammatory response in a strain and sex-dependent manner. C57BL/6J and BALB/cJ strains (male and female; n=4/group) were exposed to e-cig aerosols containing PG/VG (50:50) or menthol (Ecto 0 mg/ml and 24 mg/ml nicotine) for two hours/day for 3 days using the Scireq inExpose exposure system [Puff profile: 2 puffs/min, 70 ml puff volume]. Similarly, mice were also exposed to tobacco flavored e-cig aerosols for comparison with menthol-flavored e-cigs. Differential cell counts and inflammatory mediators were measured in bronchoalveolar lavage (BAL) fluid. Acute exposure to e-cig aerosol containing PG/VG, menthol without nicotine, and menthol with nicotine increased the total cell counts in the BAL fluid in male and female of both mouse strains. PG/VG exposure caused the highest infiltration of total cell counts in the BAL fluid. Neutrophil counts were increased in both PG/VG and menthol (0 or 24 mg/ml) exposed both male and female BALB/cJ mice. $CD4^+$ T-lymphocyte counts were altered in PG/VG and menthol with and without nicotine exposed BALB/cJ mice. Most significant changes in the differential BALF cell counts were observed in male BALB/cJ compared to C57BL/6J (male and female) mice. E-cig aerosol containing PG/VG and menthol (0 and 24 mg/ml) differentially affect inflammatory cytokines, such as MCP-1, IFNγ, KC, TNFα, RANTES, and Eotaxin in both the mouse strains compared to air group control. Further, we compared the effects of tobacco vs. menthol-flavored e-cig aerosols with and without nicotine to determine the difference in their respiratory toxicity. E-cig exposure containing PG/VG alone and menthol without nicotine induced chemotaxis such as neutrophilia and inflammatory response that may be predominantly Th1 driven. Nicotine differentially affects chemotaxis, inflammatory mediators, and increased susceptibility to immune-toxicity in menthol exposed male compared to female mice. *This study was supported by NIH R01HL135613 and U54CA228110.*

1818 Lead Exposure Modulates TNF-alpha Secretion and Arginase Activity in Macrophage J774A.1

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Lead is a potent toxic metal naturally present in the earth's crust. The extensive and continual use of lead has resulted in high pollution. The exposition to lead induces significant public health problems, alteration in the organ functioning, including dangerous effects on the immune system. The macrophages are targeted by lead, resulting in increased susceptibility to infections. The macrophages have a crucial role in immunity against intracellular pathogens and are responsible for leading the beginning (M1 phenotype), regulation and resolution of the inflammatory process and tissues reparation (M2 phenotype). The goal of this study was to evaluate the effect of lead on the macrophage's polarization to M1 and M2 phenotypes. The murine J774A.1 macrophage was exposed to 0.01, 0.05, 0.1, 0.25, 5, and 10 µg/dl lead acetate and activated with cytokines: INF γ/LPS (to M1) or IL-4/IL-13 (to M2). Lead exposure starts 24 h before the activation, and it remains present until the end of assays. Cell viability was assayed by MTT. Effects on the macrophages functionality were determined through a) the production of nitric oxide ($NO^•$) and secretion of TNF-alpha (markers of inflammation), b) the secretion of IL-10, and by arginase enzyme activity, needed to the synthesis of collagen precursors (markers of the tissue repair). At any lead concentration, was detected cytotoxic effects. On cells activated with INFγ/LPS, the lead did not alter $NO^•$ production, significantly decreased TNF-alpha secretion, whereas IL-10 levels and arginase activity were not modified. In cells activated with IL-4/IL-13, the lead did not alter levels of $NO^•$; however, it significantly increases arginase activity. Currently, the lead effect on TNF-alpha and IL-10 is being evaluated in this experimental condition. Overall, data suggest that lead partly decrease the inflammatory function of macrophages, causing a harmful modulation of the TNF-alpha secretion, while in response to the tissue repair stimulus, it increases the arginase activity and, consequently, the proline production. The mechanisms by which lead modulated the polarization of macrophages phenotypes should be further evaluated. *This study was partially funded by a grant from the Mexican Council for Science and Technology (Conacyt 152491).*

1819 Assessment of the Impact of Aerosol from Heated Tobacco Compared with Cigarette Smoke on Experimental Rheumatoid Arthritis

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Cigarette smoke (CS) is the leading environmental risk factor for many diseases and promotes the initiation as well as the aggravation of rheumatoid arthritis (RA), a chronic autoimmune disease. Alternative modified-risk tobacco products are being developed to provide substitute products for smokers who are unable or unwilling to quit. Among then, heat-not-burn tobacco products generate reduced yields of toxicants compared with cigarettes and hold great potential for reducing the harms associated with cigarette use. Here we aimed to investigate and compare the effects of CS exposure and the aerosol from heated tobacco on an experimental RA model. Male adults C57BL/6 mice were exposed to synthetic air (control), heated tobacco (heatstick HEETS, PMI) or commercial cigarettes (Marlboro, PMI) in a smoking chamber (6 animals/chamber) for 1 hour, 2 times/day, during the developmental phase of antigen-induced arthritis, between days 14 to 20. Mice were exposed to 24 cigarettes or 48 HEETS per day. Mice were injected s.c. on day 0 with 500 µg of mBSA with CFA, and boosted on day 7 and 14 with mBSA in IFA. On day 21, mice were challenged by i.a. injection of mBSA (10 µg) into the knee joint and after 24 h, inflammation was assessed by measuring edema, neutrophil migration to the joints, mechanical articular hyperalgesia, and changes in the frequencies of cells from draining lymph nodes (DLNs), spleen, plasma and bone marrow. We found that heatstick aerosol, compared with CS, demonstrated lower impact on the disease onset, observed by reduced joint swelling, mechanical articular hyperalgesia and neutrophil infiltration to the joints. Moreover, heatstick exposure did not alter the frequencies of cells in the plasma, bone marrow and spleen in comparison to the control group. However, heatstick aerosol increased the number of eosinophils in the plasma, and similar to observed from CS exposure, heatstick exposure reduced the number of cells from DLNs and the number of blasts in the bone marrow. In conclusion our data confirm the harmful effects of cigarette smoke on the RA progression and aggravation and provides novel evidence surrounding the heated tobacco products as a possible alternative to reduce the smoking-related health risks, mainly in chronic diseases.

1820 The Kinetic Direct Peptide Reactivity Assay (kDPRA): An *In Chemico* Method to Characterize the Skin Sensitization Potency of Chemicals

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While the skin sensitization hazard of substances can readily be identified using non-animal methods, the classification of potency into UN GHS sub-categories 1A and 1B remains challenging. The kinetic direct peptide reactivity assay (kDPRA) is a modification of the DPRA (OECD TG 442C) wherein the reaction kinetics of a test substance towards a synthetic cysteine-containing peptide is evaluated. For this purpose, several concentrations of the test substance are incubated with the synthetic peptide for several incubation times at 25°C. After the respective incubation time, the reaction is stopped by ad-

dition of the fluorescent dye monobromobimane (mBrB). The highly reactive and non-fluorescent mBrB rapidly reacts with unbound cysteine moieties of the model peptide to form a fluorescent complex. The remaining non-depleted peptide concentration is determined thereafter by fluorescence measurement at defined time points. Kinetic rates of peptide depletion are then used to distinguish between two levels of skin sensitization potency, i.e. to discriminate between UN GHS sub-categories 1A and 1B. In this study, we present a ring trial study of the kDPRA 24 blind-coded chemicals in seven labs. The intra- and inter-laboratory reproducibilites were 96% and 88%, respectively (both differentiating UN GHS Cat 1A sensitizers from UN GHS Cat 1B/ not classified). The extension of the kDPRA database substances to further assess the predictive capacity of the assay resulted in 88% sensitivity and 87% specificity to identify UN GHS Cat 1A sensitizers in comparison to LLNA data (167 substances) and 63% and 88% in comparison to human data (85 substances), respectively. Besides UN GHS Cat 1A identification, the kDPRA can be used as a part of defined approach(es) with a quantitative data integration procedure for skin sensitization potency assessment.

1821 Sparstolonin B, a TLR4 Antagonist is a Prime Candidate to Combat Systemic Inflammation and Restore Gut Health in GWI Mouse Models

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Gulf War Illness is a group of medically unexplained symptoms that was found to affect the veterans of the Persian Gulf War in 1991, due to exposure to toxic chemicals like Pyridostigmine bromide, Permethrin during the war theater. We have shown in our previous studies that administration of Gulf War (GW) chemicals in mouse model has resulted in gut leaching which causes portal endotoxemia that leads to upregulation of toll like receptor 4 (TLR4) receptors in small intestine. We have also found that Sparstolonin B (SsnB), a compound isolated from Chinese herb *Sparanganium stoloniferum* to be a potent TLR4 antagonist. In this study, we wanted to test in murine models whether administration of GW chemicals leads to TLR4 mediated inflammasome activation and to investigate the potential of SsnB in decreasing the inflammasome activation and restoring systemic inflammation through immunofluorescence and western blot. We found that the expression and recruitment of TLR4 in lipid rafts of intestinal epithelium was higher in GW chemical treated group which was decreased when treated in combination with SsnB. Increased expression of NLRP3/ASC and NLRP3/Caspase1 and IL-1β was observed in GW treated group which clearly indicated the activation of inflammasome and upregulation of proinflammatory cytokine suggesting inflammation. This inflammasome activation and expression of proinflammatory cytokine was significantly decreased in groups treated with SsnB and GW chemicals. We further tested our hypothesis in intestinal epithelial cells (IEC-6) the results for which corroborated with our *in vivo* results. Interestingly, we found that IEC-6 cells treated with LPS and acetylated SsnB, a pseudo inactivated drug showed results similar to LPS treated cells which provide the proof of concept that SsnB indeed was causing the effect. In conclusion, SsnB can be proposed as a therapeutic candidate against GW chemical induced intestinal inflammation and to restore gut health. *Supported by DoD Grant W81XWH1810374 and VA Merit Award I01CX001923-01 to S.C.*

1822 Effects of *In Vitro* and *In Vivo* Inorganic Arsenic Exposure on Murine Bone Marrow-Derived Macrophages

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In many parts of the world, including the United States, inorganic arsenic (iAs) contaminates groundwater used for drinking, food production, and irrigation. The World Health Organization set a 10 µg/L safety limit for arsenic in drinking water, yet still as many as 140 million people worldwide are exposed to drinking water with arsenic contamination at levels above this threshold. Eliciting a broad range of adverse health effects, arsenic is a confirmed carcinogen by the IARC and also causes increased susceptibility to infectious diseases, indicating that arsenic affects the immune system. The purpose of this study is to elucidate the effects of arsenic on the innate immune system with *in vitro* and *in vivo* exposure models. Briefly, bone marrow-derived macrophages (BMDMs) were harvested from 8-12 week old adult C57/BL6 mice. These M0-BMDMs were dosed *in vitro* with different levels of iAs (0.0001 - 1 µM) during macrophage differentiation and stimulated with LPS and IFN-γ (for "M1" macrophage activation) or IL-4 and IL-13 (for "M2" macrophage activation). Supernatant was analyzed for nitric oxide (NO) production, as well as cytokine and lipid secretion. 50-plex cytokine analysis revealed differences between iAs-exposed and nonexposed macrophages, with and without stimulation. We saw decreases in TNF with increasing iAs doses applied to M1-BMDM cultures, indicating that iAs may skew macrophages to an immunosuppressive phenotype. Exposure to iAs *in vitro* alters M1-BMDM NO production, and targeted nonpolar lipid analysis of mouse M0-BMDMs exposed to iAs had increased expression of pro-oncogenic lipids that skew macrophages to the M2 phenotype. Preliminary data shows similarly suppressed cytokine profiles replicated in an *in vivo* model where adult C57/BL6 mice were chronically exposed to 1000ppb iAs for 6-8 weeks. These data collectively suggest that iAs affects macrophage polarization, and dysregulated macrophage polarization could lead to increased susceptibility to infectious agents and cancer. Further mechanistic investigation and pathway analysis will elucidate how iAs affects macrophage function and determine whether iAs exposure makes macrophages more tumorigenic. Research such as this contributes to our understanding of the full spectrum of adverse health effects of iAs exposure and may aid in the development of therapeutics for iAs-induced diseases like cancer. *Supported by NIEHS R00ES024808 (FS) and T32ES07141 (EI, KR).*

1823 Sex Differences in Trichloroethylene-Induced CD4 T Cell Subset Differentiation in Non-Autoimmune-Prone Mice after Developmental Exposure

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Autoimmune diseases are increasing as our society has become more industrialized. While genetic predisposition is important, most autoimmune diseases are driven by environmental factors such as trichloroethylene (TCE). TCE is a halocarbon known for its use as an industrial solvent and metal degreaser. TCE has contaminated water systems, is an occupational hazard, and has been linked to several autoimmune diseases and T cell hypersensitivity disorders in humans. Developmental exposure of TCE in the drinking water increased the generation of effector/memory IFN-γ- and IL-17-secreting $CD4^+$ T cells commensurate with autoimmune hepatitis in autoimmune-prone female MRL mice relative to untreated controls. The immune enhancing effects of TCE on non-autoimmune-prone mice (e.g., C57/BL6) have not been thoroughly examined. Because TCE can also promote autoimmunity and hypersensitivity reactions in men, we also sought to determine whether developmental TCE exposure *in vivo* promoted the differentiation of pathogenic effector Th1 and Th17 and/or the suppression of non-pathogenic iTregs in both sexes of C57 mice. Naïve CD4 cells were isolated from male or female C57/Bl6 mice and incubated for 5 days to induce Th cell subsets [e.g., Th1, Th2, Th17, and inducible regulatory T cells (iTregs)]. The results demonstrated that TCE skewed Th1, iTreg, and Th17 CD4 subset differentiation in a sex-dependent manner. Specifically, IFN-γ and its major transcription factor, T-bet, were more robustly modified by TCE exposure in female mice relative to males. However, autoimmune disease pathology was not observed in any of the mice, regardless of sex. These results show that CD4s from mice that are not genetically predisposed towards developing autoimmune disease are sensitive to TCE's effects. However, the development of immune pathology likely involves other mechanisms. Further study on how TCE alters epigenetic regulation of $CD4^+$T cells during differentiation will provide meaningful mechanistic data.

1824 The 2-Hydroxyiminostilbene Metabolite of Carbamazepine or the Supernatant from Incubation of Hepatocytes with Carbamazepine Activates Inflammasomes

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Although the pathophysiology of carbamazepine-induced idiosyncratic or hypersensitivity reactions is unclear, they are presumed to be immune mediated and involve a complex interplay between drug metabolism and activation of the immune system. Reactive metabolites can cause cell damage with the release of damage-associated molecular patterns (DAMPs), which is thought to be involved in immune activation. Presumably the reason that the liver is a common target of idiosyncratic drug reactions is because it is the major site of drug metabolism and reactive metabolite formation. Inflammasomes can be activated by DAMPs, and this may be a common mechanism by which DAMPs initiate an immune response. In this study, we tested the ability of carbamazepine to induce the release of DAMPs that activate inflammasomes. Human hepatocarcinoma functional liver cell-4 (FLC-4) cells were used for bioactivation of carbamazepine. For detection of inflammasome activation we used the human macrophage cell line, THP-1 cells. We found that the supernatant from the incubation of carbamazepine with FLC-4 cells for 7 days led to increased caspase-1 activity and production of IL-1ß by THP-1 cells. In the supernatant of FLC-4 cells with carbamazepine, the heat shock protein (HSP) 60 was signifi-

cantly increased. In addition, 2-hydroxyiminostilbene, which is the metabolite of carbamazepine, activated inflammasomes of THP-1 cells. THP-1 cells whose inflammasome is activated increase high mobility group box 1 protein. These results support the hypothesis that the reactive iminoquinone metabolite can directly activate inflammasomes or it can cause the release of DAMPs from hepatocytes, which in turn, can activate inflammasomes. Inflammasome activation may be an important step in the activation of the immune system by carbamazepine, which in some patients can lead to hypersensitivity reactions.

PS 1825 Potential Therapeutic Effects of Andrographolide in Attenuating Gut-Brain Inflammatory Surge in a Mouse Model of Gulf-War Illness

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Gulf war illness (GWI) is a chronic multisymptomatic disorder, which includes metabolic syndrome, gastrointestinal (GI) disturbances and neuroinflammation, that persists till date in affected veterans. Previously we reported that butyrate administration in mice with GWI decreased metabolic abnormalities and other GWI-associated symptoms. Most recently we found that a broad-spectrum antiviral drug has potent anti-inflammatory effects on GWI pathology. Andrographolide (AG), a labdane diterpenoid produced by the plant *Andrographis paniculata*, have a broad range of anti-inflammatory properties in various cell types. Currently, our lab studies the role of AG in combating gut and brain inflammation in GWI murine models. AG was selected based on its various beneficial effects which are crucial to overcome some of the toxicity associated with prolonged use of antiviral drugs. Adult C57BL/6J mice were exposed to GWI chemicals Pyridostigmine bromide and Permethrin for a week (GWT group) and treated with AG and Ribavirin (RB). Our results showed decreased expression of tight junction proteins Zona Occludin 1 (ZO1), Occludin, which significantly decreased in mice treated with AG alone or together with RB. Also, increased expression of Claudin-2 was observed, which is a signature of "leaky gut" in GWT models. Pro-inflammatory cytokine MCP1 expression was markedly decreased with RB, AG and combination of both in small intestine. These results indicate that AG alone or together with RB can provide increased protection from gut leaching in GWI mice model. Furthermore, our results showed that AG either individually or with RB significantly decreased the levels of CD11b, while increasing the levels of BDNF and blood brain barrier tight junction protein Claudin-5. This result clearly indicates that AG either singly or in combination had a significant impact in decreasing gut-associated inflammation and neurotoxicity and can be proposed as a potential therapeutic agent in GWI patients. *This study was supported by DoD Grant W81XWH1810374 and VA Merit Award I01CX001923-01 to S.C.*

PS 1826 Single Cell Transcriptomics Reveals TCDD-Mediated Decrease in Gene Expression Associated with Erythropoiesis and Ribosomal Proteins in Early Phase of Human B Lymphopoiesis

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Persistent aryl hydrocarbon receptor (AHR) activation impairs B cell development by mechanisms not fully understood. Single cell RNA-Sequencing (scRNA-Seq) was employed to study the effect of AHR activation by 1 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early stages (7 days of culture) of B lymphocyte development. An *in vitro* culture system supplemented with cytokines that drive B cell development from human cord blood derived $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) was used. The objective was to determine how TCDD treatment modulates gene expression in differentiating HSPCs and to identify cellular transcriptomic heterogeneity. Using machine learning algorithms, we identified distinct cellular clusters in the scRNA-Seq dataset characterized by unique gene expression patterns, defined in part by expression of the AHR transcriptional target *CYP1B1*. *CYP1B1* induction was 2-fold higher in TCDD treated vs vehicle (VH; 0.02% DMSO) group, but not all TCDD treated cells expressed *CYP1B1*, indicating cellular transcriptional heterogeneity. Interestingly, *CYP1B1* expression was observed in 30% of the cells in VH group, suggesting endogenous AHR activation. Cells with higher *CYP1B1* expression in both treatment groups were associated with higher (>3.5 fold) expression of *CD14*, suggesting CD14 as a surface marker for high *CYP1B1* expression. In total, 90 genes were differentially regulated (log fold change difference > 0.25, p value < 0.01) by TCDD treatment. TCDD downregulated ribosomal protein encoding genes (11) and genes associated with erythropoiesis including drastic reductions in *HBD*, *HBB* and *HBG2*. Irrespective of treatment, most cells expressing genes associated with erythropoiesis and ribosomal proteins expressed 4-fold lower *CYP1B1* induction, suggesting that AHR activity is antagonistic to expression of these genes. This study demonstrates the presence of AHR activity in differentiating HSPCs, allows characterization of cells that are heterogeneous in AHR activity, and shows that genes associated with erythropoiesis and ribosome formation are suppressed by AHR activation early during B cell development. Further time course studies using scRNA-Seq will elucidate TCDD's effect on the cellular developmental trajectory from HSPCs to B cells.

PS 1827 Alterations in the Mouse Skin and Gut Microbiome and Skin Integrity following Dermal Exposure to the Antimicrobial Chemical Triclosan

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It is increasingly being recognized that the microbiome plays an important role in human health. Dysbiosis of the microbiome has been shown to alter immune responses and has been associated with increased risk of allergic disease. Triclosan is an antimicrobial chemical used in the healthcare field as a high level disinfectant. In humans, triclosan exposure has been associated with an increase in food and aeroallergy and asthma exacerbation. Although not directly sensitizing, dermal exposure to triclosan has been shown to augment allergic responses to experimental allergens in mouse models. However, the impact of dermal exposure to antimicrobials, such as triclosan, on the microbiome and skin integrity is unknown. This study investigated the impact of dermal exposure to triclosan on the skin and gut microbiome and on the skin integrity in mice. Mice were dermally exposed to 2% or 3% triclosan or acetone vehicle control once daily for either 7 or 28 consecutive days. Swabs were used to collect skin commensal bacteria prior to exposure and over the course of the exposure period. Following the final triclosan exposure, skin was collected to assess expression of skin integrity genes and fecal pellets were collected to assess gut commensal bacteria. Following bacterial DNA extraction from skin swabs and fecal pellets, composition of the skin and gut microbiota was determined by 16S rRNA gene sequencing. Triclosan exposure decreased the relative abundance of Proteobacteria and increased the abundance of Firmicutes, specifically the families Lachnospiraceae and Ruminococcaceae, on the skin. Triclosan exposure also led to increased abundance of Lactobacillaceae in the gut. Seven days of triclosan exposure altered the expression of two skin integrity genes, filaggrin 2 and keratin 14, in the skin. Taken together, dermal exposure to triclosan altered the composition of commensal bacteria in both the skin and gut of mice and altered the expression of skin integrity genes, suggesting that triclosan can both induce dysbiosis of the microbiome and alter skin integrity and that this may contribute to the observed alternations in immune function.

PS 1828 Effects of THC and CBD Treatment on IL-1β Secretion in TLR7- and TLR8-Stimulated Monocytes from HIV-Negative and HIV-Positive Subjects

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Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are two immune modulating cannabinoids present in *Cannabis sativa*. THC is the primary psychotropic constituent, acting on CB1 receptors in the central nervous system and CB1 and CB2 receptors on immune cells. CBD, is non-psychotropic and possesses low affinity for both CB1 and CB2 receptors. The primary molecular target by which CBD mediates biological activity has yet to be identified. Cannabis is commonly used by human immunodeficiency virus (HIV)-infected individuals, with a prevalence of about half of those afflicted. Previous research has shown that THC and CBD can have immunosuppressive and anti-inflammatory properties. Here we report on the immune modulating activity of THC and CBD in activated monocytes, when stimulated through various toll-like receptor (TLR) pathways. The objective of this study was to determine the effects of THC and CBD on monocyte IL-1β secretion in HIV- and HIV+ individuals when activated through TLR7 and TLR8 pathways. When activated through TLR7 by R837, monocytes isolated from HIV- and HIV+ donors showed similar IL-1β secretion profiles. THC treatment in combination with R837 resulted in a concentration-dependent decrease in monocyte IL-1β secretion, while CBD augmented secretion. Stimulation through TLR8 by ssRNA40, also showed a concentration-dependent decrease in IL-1β secretion from monocytes isolated from both HIV- and HIV+ donors. Interestingly, CBD

had no effect on IL-1β secretion in ssRNA40 activated HIV- monocytes, while modestly decreasing secretion in HIV+ monocytes. In summary, CBD exhibited differential effects on monocyte-derived IL-1β depending on whether the monocytes were activated via TLR7 or TLR8, where as THC suppressed IL-1β responses to both TLR7 or TLR8 activation. *Supported by NIH R01-DA047180.*

1829 Regulation of STAT3 but Not STAT1 Signaling in Human Naïve B Cells by TCDD-Mediated Alteration of the SHP-1/PP2a Signaling Axis

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant formed as a by-product of organic synthesis and incineration of organic materials. TCDD has potent immunotoxic effects in B lymphocytes resulting in decreased cellular activation and suppressed IgM secretion following activation with CD40 ligand. Previous work from our lab demonstrated that TCDD treatment of naïve human B cells resulted in significant increases in the levels of the tyrosine phosphatase SHP-1 which, corresponded with suppression of IgM secretion. STAT proteins are critical mediators of immune cell activation and effector function via their phospho-regulation. STAT3 is critical for B cell activation and secretion of immunoglobulins (Ig). STAT3 can form homodimers or heterodimers with STAT1, another critical mediator of interferon mediated immune cell signaling and translocate to the nucleus following phosphorylation as a result of cytokine receptor signaling. We hypothesized that TCDD-mediated increases in SHP-1 could result in decreased STAT3 tyrosine phosphorylation. While we found no alteration of STAT3 or STAT1 tyrosine phosphorylation, there were significant reductions in levels of STAT3 but not STAT1 serine phosphorylation as early as 12 hours following activation. These results corresponded to decreased STAT3-mediated gene transcription and increased PP2a activity, a serine specific phosphatase, which is known to be regulated by SHP-1. Lastly, pharmacological inhibition of SHP-1 phosphatase activity restored IgM secretion and STAT3 serine phosphorylation in TCDD-treated B cells while decreasing PP2a activation. Together, these data highlight a potential mechanism for TCDD suppression of Ig secretion and demonstrate the potential of inhibition of SHP-1 phosphatase activity as a means to reverse this effect in primary human B lymphocytes. *Support in part by R01ES002520.*

1830 Thirty-Day Toxicological Assessment of Perfluoroether Acids (PFEAs)

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Perfluoro-2-methoxyacetic acid (PFMOAA), perfluoro-2-methoxypropanoic acid (PFMOPrA), and perfluoro-4-methoxybutanioc acid (PFMOBA) are per- and polyfluoroalkyl substances (PFAS) structurally considered perfluoroether acids (PFEAs) as they contain an oxygen in between the carbon chains. Some PFEAs have replaced the longer carbon chain compounds perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Recently, these compounds have been identified in surface waters used as drinking water sources for several communities within the state of North Carolina. While the toxicity of PFOA and PFOS have been well characterized in animal models and epidemiological studies of humans, to our knowledge, no toxicological data exist in the publicly available literature for PFMOAA, PFMOPrA, and PFMOBA. Therefore, the present studies sought to describe signs of toxicity following 30 days of exposure to various oral doses of these compounds. Adult male and female C57BL/6 mice (6-8 weeks old) were exposed by gavage once/day for 30-days to PFMOAA at 0mg/kg, 0.00025mg/kg, 0.025mg/kg, or 2.5 mg/kg, or to PFMOPrA or PFMOBA at 0 mg/kg, 0.5 mg/kg, 5 mg/kg, or 50 mg/kg. Endpoints collected included in-life observations, organ weights, immunophenotype of lymphoid organs, and liver peroxisome proliferation. At doses administered, no differences were detected in terminal body weight, liver, spleen or thymus weights. Some shifts in immune cell populations were observed within male and female spleen and thymus in response to PFMOAA and within female thymus in response to PFMOBA. Male spleen B and NK cells were increased by ~83% and ~97%, respectively in response to PFMOAA at 2.5 mg/kg. PFMOBA induced a ~50% increase in peroxisome proliferation in the females at 50 mg/kg, while PFMOAA induced a ~25% increase in peroxisome proliferation in females exposed to 2.5 mg/kg. Exposure to PFMOPrA did not affect any of the measured parameters at any of the administered doses. These results indicate that these "understudied" PFAS discovered in North Carolina have toxicological potential that require additional investigation. Ongoing studies are evaluating the ability of these compounds to affect immune function, a sensitive endpoint associated with exposure to PFAS.

1831 Difference in Cellular Immune Functions between Broiler Chicken Husbandry and Grape Orchard Workers

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Exposure to organic dust, especially in animal husbandry environment, is recognized to affect farmer's health associated with respiratory illness. Orchard farm workers are exposed to various toxic substances including organic dust, agrochemicals, or physical hazards such as ultraviolet irradiation, but little is known about the immune modulation to orchard farmers. This study aimed to compare the general immunity of orchard farmers with broiler chicken farmers. Blood taken from the chicken or grape orchard workers was subjected to plasma IgE and IgG subclass measurements. Isolated blood mononuclear cells (PBMC) were stimulated and cytokine production was measured. Respirable dust levels were determined at each indoor (chicken farms) or outdoor workplace (grape orchards). Fifteen male and 4 female orchard workers (age: 65±8) and 11 male and 6 female chicken husbandry workers (age: 59±12) participated. Respirable dust level was higher in the chicken confinement buildings (mean 119 μg/m^3) than the grape orchard workplace (61 μg/m^3). Regarding the skewedness toward type-2 helper T cell immune reactivity, interleukin (IL)-4:interferon (IFN)-γ and IL-13:IFN γ ratios were significantly higher to the orchard workers than the chicken farmers. Level of IgE, frequently cited as a humoral allergic marker, was significantly higher to the orchard farmers than the chicken farmers. Proportions of $CD8^+$ cytotoxic T cell, $CD3^-CD16^+CD56^+$ natural killer cell, and $CD19^+$ B cell were lower to the orchard workers than the chicken husbandry workers. Considering the differences in the immune parameters, and no significant difference in age and gender composition, immune homeostasis are apparently disturbed in the orchard farmers compared to the chicken farmers. Further investigation with enough number of study subjects is needed for generalization of those findings. *Supported by Rural Administration Agency PJ014269022017.*

1832 Effects of IL-4 Administration or Zinc Supplementation on Thymic Fat in Zinc Deficient Rats

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Nutritional zinc deficiency induces immune dysfunctions including inflammatory diseases. We previously demonstrated that the inflammation induced by zinc deficiency can be inhibited by IL-4 administration or recovered by zinc supplementation. Furthermore, the inhibition of this inflammation was also associated with reduced thymus atrophy. In the current study, we explored the involvement of PPAR-γ expression in thymus atrophy (thymic fat) in zinc-deficient rats. In addition, we examined whether the factors related to T cell maturation were affected by zinc deficiency. Five-week-old male rats were fed a standard diet (17 g/day), and two groups were fed a zinc-deficient diet (n = 7 each). Each group was also injected with saline or IL-4. Another group of rats were fed a zinc-deficient diet for 6 weeks and returned to the standard diet for 4 weeks thereafter. After the dietary manipulation, qRT-PCR was performed to estimate *PPAR-γ, IL-7, SCF*, and *TSLP* mRNA expression levels. Immunohistochemical staining for *PPAR-γ* was carried out on thymus tissue sections utilizing antibodies. The thymus was also pathologically examined by oil red O stain. Positive cells and areas were quantitated by image analysis. The number of PPAR-γ positive cells, PPAR-γ mRNA expression, and the oil red O positive area in the thymus was significantly higher in the zinc-deficient group compared to the others. mRNA expression of IL-7 in the thymus was significantly lower in the zinc-deficient group than in the other groups. However, mRNA expression levels of SCF and TSLP in the thymus was comparable among all groups. The number of PPAR-γ positive cells, PPAR-γ mRNA expression and thymic fat in zinc-deficient rats were increased. Therefore, the PPAR-γ appear to relate to thymic fat. Among factors involved in T cell maturation, IL-7 expression in the thymus of zinc-deficient rats was decreased. The decrease in IL-7 mRNA expression may be due to thymic fat. Also, the IL-4 administration or zinc supplementation did not increased the PPAR-γ expression, and thymic fat was not caused. It suggested that IL-4 administration or zinc supplementation inhibits thymic fat in zinc-deficiency.

1833 The Effects of Graphene and Other 2D Nanomaterials on Immune Toxicity

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Few-layer-graphene and other 2D-nanomaterials such as molybdenum disulphide have been gaining popularity in various biochemical and industrial uses as demand for such versatile and ground-breaking materials increases. It is therefore pertinent to investigate the toxicological impact of such materials on immune cells, which are the body's main line of defence. We focus on human peripheral blood monocyte-derived macrophages cultivated using both GM-CSF and M-CSF to better understand the effect of these materials, and have found minimal detriment to cell viability and macrophage surface marker expression, as well as an increase in autophagic flux using LC3-II western blotting. We also observed heterogeneity in material uptake using phase-contrast microscopy, which could augur patient-to-patient variability in cumulative exposure. With the results of our research, we hope to better shape the design of future graphene- and 2D-based nanomaterials for improved immune compatibility.

1834 Metabolic Syndrome Enhances Nanoparticle-Induced mTOR Signaling via Alterations in the Biocorona

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Nanoparticles (NPs) interact with biomolecules forming a biocorona (BC) in biological environments. The BC impacts biological responses affecting NP biomedical applications. Metabolic syndrome (MetS) is a prevalent condition and results in the formation of unique BCs. In this study, we examined differential cell signaling that may occur in MetS by using the BC. Rodent cell lines (rat endothelial cells and mouse macrophages) and human relevant Fe3O4 NPs without or with BCs were utilized. NPs were incubated in water, 10% normal rat serum, or 10% MetS rat serum to form BCs. Endothelial cells were exposed to NPs for 3, 12, or 24 h and differential cell signaling was discovered using a RNA-Seq approach. Functional enrichment of the differentially expressed genes identified differential activation of pathways including DNA damage repair, oxidative stress, inflammation, and mTOR signaling. Examination of mTOR signaling demonstrated MetS BC enhanced phosphorylation of AKT and elevations in total and phosphorylated mTOR. Markers of apoptosis (cleavage of caspase-3 and cytochrome c levels) were increased only in cells exposed to NPs with a MetS BC. mTOR signaling induces apoptosis via inhibition of autophagy. Macrophages demonstrated decreased gene expression of LC3, a marker of autophagy, only in cells exposed to NPs with a MetS BC. Treatment with rapamycin, an inhibitor of mTOR, inhibited changes in LC3 expression. To understand up-stream regulation of mTOR, we utilized pharmacological inhibitors of scavenger receptors. CD36 was determined to be the primary facilitator of NP internalization and is increasingly expressed due to MetS. A CD36-specific ligand enhanced mTOR gene expression similarly to NPs with a MetS BC. A number of processes down-stream of mTOR (insulin sensitivity and lipogenesis) were uniquely altered by the MetS BC. Together these findings demonstrate that the formation of a BC within MetS may result in enhanced mTOR signaling due to increased scavenger receptor interactions leading to exacerbated responses and progression of MetS-associated diseases.

1835 miRNAs and Dendritic Cell Activation in Allergic Contact Dermatitis

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Allergic contact dermatitis (ACD) is an immunological mediated inflammatory disease and is one of the most common occupational disease in industrialized countries. Chemical allergy is of considerable importance to the toxicologist, who has the responsibility of identifying and characterizing the allergenic potential of chemicals and estimating the risk they pose to human health. While incredible progresses have been made in the development of non-animal tests, currently it is not possible to estimate the sensitizing potency of chemical allergens. The main aim of this project was to investigate if contact allergens of different potency are able to differently modulate miRNA expression, using only *in vitro* methods. Recent findings indicate that miRNAs may determine reprogramming of gene expression in target cells. Although ACD has been studied extensively, there are few studies conducted to investigate miRNA expression. Based on our preliminary results, and the working hypothesis that allergens of different potency induce different cellular activation, two contact allergens of different potency were selected: 2,4-dinitrochlorobenzene (DNCB - extreme) and imidazolidinyl urea (IMZ - weak), respectively extreme and weak contact allergens. THP-1 and subsequently, differentiated THP-1 (mature dendiritc cells - mDCs) cells were used as cellular lines. Additional experiments have been done with the microvesicles (MVs) released from THP-1 cells, always after exposure to extreme and weak contact allergens. Specific Array Panels for miRNAs screening were performed to evaluate the up and down regulation of these after cells treatment. Target genes and protein have been finally identified and confirmed. Results obtained shown a different miRNAs regulation induced by contact allergens of different potency in THP-1 cell. In particular, results obtained evaluating 6 selected miRNAs, based on literature research and miRNAs target databases, suggest an important role played by miR-27a-3p and miR-223-3p. From analysis of data obtained after differentiation and maturation of THP-1 cells, it is interesting to notice that immature dendritic cells (iDCs) exposed to the extreme contact allergen DNCB were able to induce a modulation of miRNA similar to mature dendritic cells (mDCs) obtained starting from THP-1 with the addition of a cocktail of maturation factors (with the exception for only one miRNA - 27a-3p). These results support previous data obtained in our laboratory that demonstrate that extreme contact allergens lead to a full maturation of iDCs.

1836 Tcdd-Mediated Upregulation of FasL on B Cells Triggers Apoptosis in T Cells in EAE

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Previous results from our laboratory showed that TCDD can suppress the autoimmune disease experimental autoimmune encephalomyelitis (EAE). Specifically, TCDD suppressed T cell immune function in the spleen, which correlated with decreased neuroinflammation and clinical scores. The goal of this project is to determine if TCDD-treated B cells contribute to decreased T cell function. Our hypothesis is that TCDD will induce Fas ligand (FasL) regulatory B cells in EAE, which could induce apoptosis in Fas-expressing cells, such as T cells. We developed the JC-1 assay, which measures mitochondrial membrane potential as an indicator of cell viability. Optimization was done *in vitro* using FCCP as a positive control. We then conducted studies using TCDD-treated lymphocytes, B cells or IgM-depleted B cells mixed with target T cells that were prestained with CD4. The rationale for depleting IgM for some B cell preparations is that TCDD upregulates FasL to a greater degree on follicular B cells, which express more IgD than IgM. We first used lymphocytes isolated from spinal cord following an 18-day EAE disease course with 30 µg/kg TCDD given i.p. on day 1 but did not detect apoptosis in CD4+ T cells in the presence of EAE plus TCDD-treated spinal cord lymphocytes. We then used cells isolated from spleen following an 18-day disease course with 30 µg/kg TCDD given orally over 12 days at 2.5 µg/kg/day. We mixed EAE plus TCDD-treated splenocytes, purified B cells or IgM-depleted B cells with CD4+ T cells. In two separate replicates of the experiment, mitochondrial membrane potential in the CD4+ T cells was lower in the presence of TCDD-treated cells as compared to corn oil vehicle-treated cells. The decrease in mitochondrial membrane potential in CD4+ T cells was highest in the presence of EAE plus TCDD-treated IgM-depleted B cells (7-33% decrease ratio of JC-1 aggregates to monomers). Together these results demonstrate that the mechanism by which TCDD suppresses T cell function involves its ability to induce FasL on B cells, which triggers apoptosis in T cells. *Supported by NIH R15ES027650.*

1837 Effect of TCDD on Antibody Production in Various Anatomic Locations in Experimental Autoimmune Encephalomyelitis

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The prototypical AhR ligand, TCDD, has been well characterized to be immunosuppressive. It has been determined that TCDD robustly attenuates the autoimmune disease experimental autoimmune encephalomyelitis (EAE), a mouse model to study multiple sclerosis. The purpose of this study was to evaluate IgG production in different anatomic locations including spleen, lymph node, bone marrow, spinal cord, and blood. In each location, specific B cell populations, including CD19, B220, and CD5, were analyzed in mice induced with EAE disease and treated with TCDD. CD19 and B220, an isoform of CD45, are B cell markers expressed at all stages of B cell development but lost upon plasma cell differentiation. CD5 is an indicator of one population of innate B cells. To evaluate antibody production in the B cell populations of TCDD-treated mice, disease was induced and 2.5 µg/kg/day of TCDD was orally administered for 12 days, for a total of 30 µg of TCDD. It was hypothesized that TCDD would downregulate IgG production in all anatomic locations. TCDD was very effective in suppressing MOG-specific IgG in serum. Overall, IgG was suppressed by TCDD in splenocytes. Interestingly, IgG was most affected by TCDD in CD19-, B220-, and CD5-negative populations. TCDD

did not affect IgG production in bone marrow- or lymph node-derived B cells, but TCDD did suppress IgG production in B cell populations in the spinal cord. In conclusion, these data show that TCDD's target tissues at end-stage disease are spleen and spinal cord. These results provide valuable insight into the mechanism of immunotoxicity of TCDD. In addition, the findings contribute to the understanding of how AhR ligands affect EAE and may help us develop a less toxic treatment for autoimmune disease. *Supported by NIH R15ES027650.*

1838 $OANO_2$ Increases the Likelihood of Survival and Resolution Signaling in Response to Bleomycin-Mediated Lung Injury

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Nitro-oleic fatty acid ($OANO_2$), a potent electrophile, is endogenously found in humans and modifies cysteine residues via Michael addition to alter protein function. It reduces inflammatory activation in the cardiovascular system, and potentially the lung. Intratracheal bleomycin (ITB) is a model of pulmonary inflammation, resolution, and fibrosis. Earlier work showed a loss of resident alveolar macrophages (AMs) and activation of interstitial macrophages (IMs) in the early phase of the response to ITB (7 days) that was abrogated by $OANO_2$. Here, we examined the effect of $OANO_2$ on the resolution phase of the ITB response. ITB (3 U/kg) was administered to animals while $OANO_2$ was delivered via osmotic pump (1 nmole/g/hr). Bronchoalveolar lavage (BAL) and lung tissue were collected at 17 days post ITB. Our prior studies have shown that $OANO_2$ alters macrophage activation at 7 days post ITB. Therefore, we examined both BAL and tissue macrophage phenotypes in response to ITB with and without $OANO_2$ treatment. In the BAL, resident AMs (CD45+, SiglecF+, F4/80+, CD11c+) were lost 7 days post ITB (95 ± 3.9% vs 49 ± 3.9%*) but recovered by 17 days (68 ± 3.5%*). $OANO_2$ preserved resident AMs at 7 days (69 ± 3.9%*) and increased recovery at 17 days (73 ± 3.8%*). IMs show a similar pattern of activation and resolution. ITB increases the proportion of mature IMs (CD11c+) expressing both Ly6C (43 ± 3.7% vs 13 ± 3.4%*) and mannose receptor (MR) (28 ± 3.6% vs 5 ± 3.4%*) at 7 days; returning closer to control at 17 days (Ly6C+ 16 ± 2.4%; and MR+ 9 ± 1.2%). $OANO_2$ reduces activation at 7 days post ITB (Ly6C+ 27 ± 2.4%#; and MR+ 13 ± 3.9%#), but perpetuates activation at 17 days (Ly6C+ 28 ± 5.1%#; and MR+ 10 ± 2.3%). These observations are consistent with $OANO_2$ driving an increased level of resolution. This is confirmed within mesenchymal stem cells (CD45-, CD31-, Sca1+), where ITB increases fibrotic signaling (CD44+) at 7 days (70 ± 4.7% vs 26 ± 4.5%*); while the addition of $OANO_2$ reduces the early expression of CD44 (58 ± 4.3%#) but potentiates activation at 17 days (CD44+ 69 ± 5.3%#; and CD90+ 23 ± 1.7%#). Mice administered ITB lost a significant amount of bodyweight compared to controls; $OANO_2$ mitigated this loss at 7 days (-4.0 ± 0.3% vs 0 ± 0.5%#); and this weight loss continued to 10 days post ITB. At this time point mice either stabilized in weight or did not survive. Importantly survival rates were higher in $OANO_2$ treated animals (80% vs 35% #) at 17 days post ITB. Histological findings in animals sacrificed revealed signs of inflammation, epithelial destruction, and proteinaceous deposits. These findings suggest that $OANO_2$ administration increases the likelihood of resolution and survival (* $p<0.05$ vs control; # $p<0.5$ vs ITB).

1839 Role of Arsenic in Modulating Ligand-Induced Microglial Neuroinflammatory Response

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Microglia plays an important role in various neuroinflammatory diseases, where it gets activated and secrets proinflammatory cytokines, leading to neuronal damage. Microglial activation status can be modulated by various environmental factors like arsenic, known to induce immunosuppression. Lipopolysaccharides (LPS) is a ligand for TLR-4 involved in NF-kB signaling. It can induce microglial activation where Poly (ADP Ribose) Polymerase PARP1 is involved. PARP1 is a zinc finger protein containing C3H1 motif and arsenic has been reported to selectively bind to zinc finger motifs especially C3H1 and C4. We investigated the effect of arsenic on ligand-induced neuroinflammation and the involvement of PARP1 in this event. Male Balb/c mice were exposed to 0.38 mg/kg bd. wt. sodium arsenite ($NaAsO_2$) for 30 days following intraperitoneal 0.33 mg/kg LPS exposure for 24 hours. *Ex vivo* microglia isolation was done to check the level of proinflammatory cytokines through multiplexing. With it, microglia cell line (N9) was also exposed with 500nM $NaAsO_2$ and 1.2 µM $ZnCl_2$ for 24 hours followed by 100 ng/mL LPS for 6 hours and multiplexing was performed to check the levels of pro-inflammatory cytokines. Arsenic exposure decreases LPS-induced microglia inflammation as well as LPS induced Poly ADP Ribosylation by inhibiting PARP1. This inhibition of PARP1 activity by binding with arsenic suppresses p65 phosphorylation and blocks microglia activation. Supplementation of Zinc in microglia cells neutralizes the arsenic-mediated reduction in LPS-induced proinflammatory cytokines production that confirms the involvement of PARP1 in this event. Additionally, *in silico* analysis reveals that ATR and Chk1 can be involved in the p65 phosphorylation pathway. Thus, pre-treatment of ATR/Chk1 inhibitor reduces p65 phosphorylation following LPS stimulation, confirms the involvement of these two molecules in ligand-induced neuroinflammation. Therefore, our study will help in the development of new therapeutic strategies for neuroinflammatory diseases and unveil the mechanism of arsenic mediated suppression of ligand-induced neuroinflammation by finding the missing link between PARP1 and NF-kB signaling and raise the possibility of using arsenic for its therapeutic effect at lower doses.

1840 Inhibition of the Respiratory Burst by Six Per- and Polyfluoroalkyl Substances (PFASs)

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Per- and polyfluoroalkyl substances (PFASs) are a class of almost 5,000 anthropogenic compounds used in the production of non-stick coatings, food wrappers, water- and stain-resistant fabrics, and fire-fighting foams. Due to their ubiquity, persistence, and mobility in the environment, between 95% of 99% of all Americans have detectable levels of PFASs in their blood. Exposure to PFASs has been linked to cancer, thyroid disease, and impaired adaptive immune system responses. However, the impact of PFASs on the innate immune system remains unknown; should the innate immune system be impaired, the host may be susceptible to infectious or neoplastic disease. In order to investigate this, larval zebrafish and human neutrophil-like cell lines were exposed individually to six PFASs that have been detected in the water and air in Fayetteville, North Carolina: PFOA, PFOS, PFHxA, PFHxS, GenX, and fluoroether E1. Initially, we performed range-finding studies to determine doses that were non-teratogenic to zebrafish and non-cytotoxic to human cells. Doses for which no teratogenicity or cytotoxicity were observed served as the highest doses tested in subsequent assays. We then measured the respiratory burst as a functional readout of innate immune function. The respiratory burst is the process by which phagocytes, such as macrophages and neutrophils, produce microbicidal reactive oxygen species to fight infections. Range-finding studies for developmental toxicity and cytotoxicity identified PFOA and PFOS as developmentally toxic to larval zebrafish, but none of the tested PFASs were cytotoxic. In the respiratory burst assay, PFOS, PFHxA, and E1 suppressed the respiratory burst *in vivo*. However, *in vitro*, all of the PFASs that were tested suppressed the respiratory burst. This may indicate that, while PFASs are able to directly impair innate immune function *in vitro*, certain PFASs may target other organ systems *in vivo*, resulting in no observable immune dysfunction. Future studies plan to explore this hypothesis, whether PFAS-induced immunotoxicity is differentially influenced by chronic or acute exposure, and whether exposure to PFASs confers susceptibility to infectious disease.

1841 Effects of Two Food Additives, 3-HT and BHT, on OVA-Elicited Food Allergy in Mice

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There has been a steady rise in the prevalence of food allergy for the past couple of decades, however the underlying cause has not been identified. Many factors contribute to food allergies, including environmental chemical exposure, genetics, microbiota, etc. Recent findings show that there could be a link between a common food additive, tBHQ, and food allergies, which suggests the need to test other food additives. In our study, we investigated if butylated hydroxytoluene (BHT) and 3-hydroxytyrosol (3-HT), play a similar role as tBHQ in an OVA-sensitized mouse model. Female BALB/c mice (4 weeks old) were given a control, BHT (0.0014%), or 3-HT (0.0014%) diet and exposed to OVA transdermally once per week for four weeks during the sensitization phase. Sensitization to OVA was assessed by the rise in OVA-specific IgE and IgG1 by ELISA. Whereas BHT and 3-HT decreased the IgE response to OVA, IgG1 levels were similar between all the diets during the sensitization phase. Upon oral challenge, mice were monitored for systemic anaphylaxis, hypothermia shock response (HSR) and mast cell protease (mMCP)-1 response. Systemic anaphylaxis clinical symptoms were quantified. Mice on the 3-HT and BHT diets had less severe clinical signs of anaphylaxis. BHT and 3-HT diminished the decrease in body temperature with a more rapid return to normal temperature as compared to control diet. Mouse mast cell protease-1 (mMCP-1) levels from BHT, 3-HT and control group were determined through ELISA, indicating decreased mast cell degranulation in the BHT and 3-HT groups. In addition, flow cytometry showed T helper 2 (Th2) cells (CD4+ IL-4+) population in splenocytes from the 3-HT-treated group were decreased

significantly compared to control. Taken together, BHT and 3-HT may have a protective effect in regard to the OVA-elicited food allergy mouse model, in contrast to tBHQ. These results could potentially help industry decision makers reconsider which food additives to use as preservatives and will be important to mitigate the rise in food allergy.

1842 Pathway Analysis for Immunotoxicity Assessment by Co-culture of THP-1 Cells and NHEK

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To establish a skin sensitization assessment method including cell-cell interactions and chemical metabolic reactions, we integrated human monocytic leukemia cell line (THP-1 cells) into a skin model using differentiated normal human epidermal keratinocytes (NHEK). However, previous studies have shown that medium composition and the secretion from NHEK affected the expression of THP-1 cells surface marker, which is an index for assessing skin sensitization. In this study, gene expression profiling using microarray was performed to explore new indicators in the co-culture system. Human epidermis model was developed on the cell culture insert according to the protocol of EPI-KIT (J-TEC). These culture inserts transferred into well of 24-well plate which were seeded with THP-1 cells at density of 1×10^6 cells/well. As the medium for co-culture, THP-1 cells culture medium and the mixture of THP-1 cells culture medium and keratinization induction medium at the ratio based on seeding number of each cells (the mixed medium) was used. 2,4-Dinitrochlorobenzene (DNCB) as a test substance was exposed to THP-1 cells through a human epidermis model, and total RNA was isolated from both cells after exposure for 18 hours and analyzed transcriptome changes using with Clariom™ S Human Array (Thermo Fisher Scientific). The pathway analysis tool, Wiki Pathways, was used to examine the immune response of THP-1 cells by DNCB treatment after clarifying the effects of medium composition and secretion from NHEK. Several pathways were activated by changing from THP-1 culture medium to the mixed medium however there were no pathways significantly induced by co-culture with NHEK. Regardless of medium composition and culture condition (co-culture or pure culture), DNCB treatment activated MAPK signaling pathway which mediate CD40/CD54/CD86 expression[1], and VEGFA-VEGFR2 signaling pathway which regulate ICAM-1 protein expression via TNF-α[2]. Our results ware suggested that skin sensitization evaluation using the gene expression as an index is possible in co-culture system. *References: 1) Miyazawa, M., Ito, Y., Kosaka, N., Nukada, Y., Sakaguchi, H., Suzuki, H., & Nishiyama, N. (2008). Role of MAPK signaling pathway in the activation of dendritic type cell line, THP-1, induced by DNCB and NiSO4. J. Toxicol. Sci., 33 (1), 51-59. 2) Peeradech, T., Steven, J,H., Kanokpan, W., & David, O,B. (2014). TNF-α-induced ICAM-1 expression and monocyte adhesion in human RPE cells is mediated in part through autocrine VEGF stimulation. Mol. Vis., 20, 781-789.*

1843 Regulation of Ubiquitin Specific Peptidase 2 by Farnesoid X Receptor in Glioblastoma NCI-60 Cell Lines

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Glioblastoma multiforme is the most common primary brain tumor in adults. It is extremely aggressive and one of the least treatable types of cancers. Although treatment options include surgery, chemotherapy, and radiotherapy, the median survival is 14-15 months after diagnosis. With no obvious cause and little progress in development of new therapies, there is a clear need to identify new potential targets. Farnesoid X Receptor (FXR) is a nuclear receptor predominantly expressed in the liver and intestine, where it is has been reported to be critical in maintaining bile acid homeostasis and suppress hepatocellular carcinoma through anti-inflammatory activity and repair of liver injury. Our laboratory has previously found FXR expressed in the brain. Specifically, in mouse and human astrocytes FXR regulates the inflammatory response to pro-inflammatory cytokines. Additionally, we evaluated the expression of FXR in six NCI-60 glioblastoma cell lines, one immortalized glioblastoma cell line (ATCC U118), and primary human astrocytes. Basal levels of FXR were measured by qPCR and immunofluorescent staining, indicating the presence of this gene product and protein in glioblastoma and human astrocyte cells. When these cells were treated with an FXR agonist (10μM WAY 362450), significant induction of the validated downstream target gene small heterodimer partner (SHP) occurred. Further, Ubiquitin Specific Peptidase 2 (USP2), a deubiquitinating enzyme with a complex role in tumorigenesis, was increased following FXR agonist treatment in the glioblastoma and primary human astrocyte cell lines. qPCR and immunofluorescent staining revealed a significant increase in USP2 mRNA (~1.5-2.1 fold) and protein (~1.2-2.0). These data further solidify the presence of functional FXR in human astrocytes and glioblastoma, identifying it as a desirable target for further research.

1844 Metallothionein, a Small Stress Response Protein, Serves as a Co-Stimulatory Molecule for Lymphocyte Activation

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Metallothioneins (MTs) are stress response proteins that are highly inducible by various agents including glucocorticoids, divalent heavy metals, reactive oxygen species, and acute phase cytokines. 33 mol% of MT's amino acid composition are cysteines that provide sulfhydryl groups responsible for metal binding and scavenging reactive oxygen species. Due to its rapid induction in response to acute phase cytokines, MT has been suggested to have potential role(s) in inflammatory microenvironments. Although the intracellular roles of metal homeostasis and free-radical scavenging are typically cytoprotective in inflamed tissues, MT that is released into the extracellular environment may exacerbate local inflammatory activities as a consequence of its chemotactic activities. We have shown that MT also has a modest proliferative effect on B cells and can increase the pool of proliferating cells up to 4-fold when co-administered with bacterial lipopolysaccharide (LPS). This suggests MT may be a costimulatory signal that enhances the vigor of responses to other pro-inflammatory stimuli. Biotin-labeled MT can be detected on the surface of murine lymphocytes by fluorophore-conjugated streptavidin, suggesting MT may provide a costimulatory signal for proliferation by interacting with membrane receptor(s). These interactions may include interactions with chemokine receptors, as shown by downstream signaling and GPCR-specific screening. The synergistic effect of MT on LPS-induced B cell proliferation is not diminished by administration of MT-specific monoclonal antibody, suggesting the costimulatory effect of MT does not require direct activation of a plasma membrane receptor. The antioxidant activity conferred by the free thiols within MT's structure may serve to manage the local redox environment for redox-sensitive surface molecules. The antioxidant glutathione had no effect on proliferation when administered at a dose representing the same reactive thiol molarity as MT used in these studies, suggesting the costimulatory effect of MT is also not solely due to its ability to provide a reducing extracellular environment. Determining MT's mechanism of action in lymphoproliferation may illuminate the role of endogenous MT in the context of the chronic inflammatory stress that can be associated with autoimmune disease and thus lead to novel therapeutic opportunities.

1845 Spatial Dependence of Cytokine and Phosphoprotein Distributions following Exposure to Localized *Staphylococcus epidermidis*

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The spatial gradients associated with localized infection are not currently well defined, despite the fact that these infections remain a serious complication in clinic. *Staphylococcus epidermidis* is often implicated in these medical device-related infections, and it can lead to failure rates up to 50%. While current surgical treatments rely on understanding the margins of these localized infections, the tissue-level inflammatory response to *S. epidermidis* has not been well investigated spatially. Defining the spatial inflammatory immune response may aid future efforts to combat these persistent infections. In this study, human inflammatory cytokines and phosphoproteins were profiled in patients undergoing revision knee arthroplasty to investigate the spatial dependence of tissue-level response to infection. Samples were collected from male (N=5) and female (N=5) patients, varying in age (46-76 years) and comorbidities. Tissues were collected at seven locations, varying in proximity to prosthetic knee. Six cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-8, and MIP-1α) and four phosphoproteins (Akt, BAD, p70S6K, and ZAP-70) were able to discriminate aseptic vs. septic tissues via two-way ANOVA followed by Bonferroni's post-test ($p<0.05$). Principal component analysis (PCA) was conducted to better characterize the spatial disparities of these responses. Overall, it suggested that phosphoprotein targets (Akt, BAD, p70S6K, and ZAP-70) may be indicative of aseptic tissues, and cytokine targets (IL-1α, IL-1β, IL-4, IL-6, IL-8, and MIP-1α) may be indicative of septic tissues. The ANOVA and PCA data together indicate that there are observable spatial differences ($p<0.05$) in inflammatory tissue response at the levels of cytokines and phosphopro-

teins following localized *S. epidermidis* infection. Through the analysis, it was possible to identify regional variability and offer novel information about tissue-level disparities in localized inflammatory response.

1846 Nimbolide Abrogates Cerulein-Induced Chronic Pancreatitis by Modulating β-Catenin/SMAD in a Sirtuin 1-Dependent Way

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Chronic pancreatitis (CP) is characterized by pancreatic inflammation and fibrosis, which leads to impairment of pancreas function. The present study was designed to investigate the possible mechanisms of chronic pancreatitis and the anti-inflammatory, antioxidant and anti-fibrotic effect of nimbolide (NB), active constituent of neem tree *Azadirachta indica* in cerulein-induced CP. Effect of nimbolide was investigated on cerulein 50 $\mu g.kg^{-1}.hr^{-1}$*6 exposures^{-1}day 3 days a week for 3 weeks induced CP model in Swiss albino mice. Nimbolide (0.3 and 1 mg/kg) and sirtuin 1 (Sirt1) inhibitor, nicotinamide (200 mg/kg), were given intraperitoneally daily for 21 days. Pancreatic function was assessed by biochemical evaluation including amylase and lipase levels. The deposition of collagen in pancreatic tissue was measured by using hydroxyproline assay, Picrosirius red and Masson's trichrome staining. The expression of collagen I, α-smooth muscle actin (SMA) and β-catenin in the pancreas tissue was evaluated using immunohistochemistry and immunofluroscence. NB treatment significantly reduced cerulein-induced CP by inhibiting Wnt/β-catenin signaling pathway including β-catenin, MMP7 and GSK3β. NB treatment remarkably decreased α-SMA, TGF-β1, MMP-2, fibronectin, p-smad-2/3 expression and collagen deposition in pancreatic tissue. However, the therapeutic effects of nimbolide against cerulein-induced CP were impaired by nicotinamide treatment. The levels of pro-inflammatory and pro-fibrotic cytokines such as TNF-α, IL-1β, IL-6 and TGFβ1 were elevated by cerulein treatment; further, they were decreased by NB treatment. Additionally, NB was found to increase the expression of Sirt1 protein, which ultimately decreased the expressions of Smad2/3 and β-catenin signalling pathway proteins to attenuate cerulein-induced CP. Nimbolide attenuated cerulein-induced CP by activating Sirt1, which regulates Smad/β-catenin signaling pathways and it could be the novel therapeutic strategy for the treatment of CP associated fibrosis.

1847 Role of Aryl Hydrocarbon Receptor Ligands in Allograft Rejection

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Immune-mediated transplant rejection is a major health problem and use of strong immunosuppressive drugs in patients can lead to susceptibility to infections and cancer. Our studies are aimed at determining whether treatment with AhR ligands could affect transplant rejection. Towards this, the effect of AhR ligands, 6-formylindolo [3,2-b] carbazole (FICZ) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on allogeneic rejection of skin transplants from C3H/HeJ (H-2^k) donors onto C57BL/6 (H-2^b) recipient mice was studied. For this purpose, C57BL/6 recipient mice were treated with 10ug/kg TCDD or 50ug/kg FICZ starting 1 day prior to transplantation every 3 days for 12 days. Our studies demonstrated delayed allograft rejection in TCDD- but not FICZ-treated mice. In addition, increase in CD8+ T cells was observed in vehicle-treated graft recipient mice, which was decreased following treatment with AhR ligands. Furthermore, treatment with the AhR ligands led to decreased expression of Th1 ($CD3^+CD4^+Tbet^+$) cells and increased expression of (CD3+CD4+FoxP3+) Tregs in graft-draining lymph nodes. We next examined the effect of AhR ligands (10nM TCDD or 10uM FICZ) on mixed lymphocyte reaction cultures of spleen cells from C57BL/6 mice as responders when stimulated with mitomycin C-treated splenocytes from C3H/HeJ mice. There was an increase in the CD4+ and CD8+ T cells following treatment with AhR ligands. While there was no change in the induction of T regs *in vitro* following treatment with AhR ligands, FICZ treatment led to an increase in Tr1 (CD3+ CD4+ FoxP3- CD233+ CD49b+) cells. In conclusion, our studies suggest modulating effects of AhR ligands in transplant rejection which may be mediated by dysregulation in CD4+ T regulatory cell subsets. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.*

1848 Role of Constitutive Cyp1b1 Expression in Macrophage on Diet-Induced Obesity: Bone Marrow Macrophage Polarization and Peritoneal Macrophage Stimulation by IL-13

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Cyp1b1 has minimal expression in hepatocytes but is robustly expressed in endothelia and in mesenchymal progenitors including in bone marrow (BM). Cyp1b1 in BM mesenchymal progenitor cells (MPC) mediates suppression of BM hematopoietic progenitors by PAH within 6h. Cyp1b1 also has many such effects on endogenous mediators of inflammatory processes often in unexpected places. Thus, Cyp1b1 deletion suppresses diet induced obesity in combination with a specific set of gene responses related to growth hormone and leptin signaling. Restriction to macrophage selected by Lyz2-CRE fully reproduces systemic Cyp1b1 deletion responses. Here we characterize macrophage originating from respectively BM (BMM) and peritoneal cavity (PM) of separate sets C57Bl/6J mice (Age 6 weeks, n=3). BMM macrophage (28 percent of BM cells) were selectively expanded by M-CSF for 6 days. PM were optimally cultured from other individual C57Bl/6J mice. M-CSF expanded BMM expressed only low Cyp1b1 but substantially elevated PPARγ. IL-13 which stimulates PM via LRH-1/Nr5a2 elevated Cyp1b1 mRNA from undetectable to robust expression but with Cyp1a1 undetectable. Three other similar LRH responders (CD36, Arg1 and ym1) were highly stimulated whereas TNfα was suppressed. Responses to IL-13 were unaffected by the specific Cyp1b1 inhibitor tetramethoxystilbene or when stimulated in Cyp1b1-/- PM. M-CSF enriched BMM were characterized for M1 polarization (IFNγ-24h stimulation) and M2 stimulation (IL-4 24h stimulation). Cyp1b1 was elevated 4-fold to significant but low levels in M2 polarization by IL4, again without Cyp1a1 but not by IFNγ. Inflammatory M1 polarization by IFNγ stimulated TNFα, peaked within 3-6h and then declined. IL-1β and iNOS appeared later. The transcription factor Ciita responded linearly over 24 hr preceding increases in Ciita-targeted four H2 histocompatibility factors (H2-Ab). Cyp1b1 deletion decreased the acute stimulation of TNFα (3-6h,$p<0.05$) but not after decline at 24h. M2 polarization of BM macrophage with IL-4 was marked by rapid increases in transcription factors (IRF4, Klf4, Egr2 and Myc) and subsequently by M2 markers (Arg1, Ym1 and Fizz1) at 24h. Other IFNγ responses or IL-4 responses were unaffected by Cyp1b1 deletion. The absence of Cyp1b1 in WT M1 macrophages indicates an indirect effect. Expression changes in Cyp1b1-/- BM have been reported. Effects of Cyp1b1 on obesity are unlikely to arise from these BMM or PM. Macrophages of brain (microglia) where Cyp1b1 metabolism of estradiol is an influence provides a more likely source.

1849 Nuclear Receptor REV-ERB Alpha Augments House Dust Mite-Induced Allergic Asthma in Mice

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Allergic asthma is a chronic inflammatory disease that displays a time-of-day dependent variation in clinical symptoms and severity. Asthma attacks, particularly in response to household allergens show a time-dependence with most severe attacks occurring in the night/early morning. The molecular clock, nuclear receptor Rev-erbα plays a considerable role in immunity and inflammation. Intriguingly, the impact of allergen-induced dysfunction on molecular clock function and its role in gating the severity of lung immune-inflammatory responses during allergic asthma is not known. We hypothesize that allergen-induced disruption of REV-ERBα expression leads to irregular clock function in the lungs, altered rhythms of clock-controlled genes and augmented immune-inflammatory responses. Wild-type (BALB/c or C57BL/6J) and REV-ERBα global knockout (KO) mice were exposed to acute house dust mite (HDM) allergen model. Mice were administered with PBS (30 µl) or HDM extract (30 µg/30 µl) intra-nasally (i.n.) for 10 consecutive days and euthanized 48 hrs after the last challenge at two different time points (am vs. pm). We measured the severity of asthmatic lung phenotypes including airway inflammation, airway hyperresponsiveness, Th2 cytokines in bronchoalveolar lavage fluid, plasma IgE/IgG, mucous metaplasia, abundance of Muc5ac, Muc5b protein, expression of mucins, *Tslp* (Thymic stromal lymphopoetin), Stat3 and clock-controlled genes (CCGs) in mouse lung. WT mice exposed to HDM show reduced expression of REV-ERBα in airway epithelium associated with increased airway inflammation, airway hyperresponsiveness, elevated Th2 cytokine levels, increased plasma IgE/IgG, mucous metaplasia and increased abundance of Muc5ac/Muc5b. qPCR analysis revealed significant reduction in the expression of *Rev-erbα* and other CCGs (*Clock*, *Bmal1*, *Rev-erbβ*, *Per1*, *Per2*, *Cry1* and *Cry2*) and enhanced expression of *Nfil3*, *Tslp, Muc5ac,* and *Muc5b*

genes in the lungs. REV-ERBα KO mice displayed a significantly greater airway inflammation, enhanced activation of Th2 cytokines and mucous metaplasia compared to WT mice. These findings from acute HDM model show an important role of nuclear receptor REV-ERBα in the pathophysiology of allergic asthma in a mouse model. Thus, REV-ERBα could be used as a novel therapeutic target using selective REV-ERBα agonist to mitigate allergen induced immune-inflammatory response. *Supported by the NIH 1R01 HL142543 (to I.K.S).*

1850 The Development of Pulmonary Inflammation and Injury in a Mouse Model of Non-Alcoholic Steatohepatitis

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Non-alcoholic fatty liver disease (NAFLD) is a chronic liver condition that affects 42 million individuals in the United States, of which ~20% progress to non-alcoholic steatohepatitis (NASH). NASH is characterized by the accumulation of fat in the liver and persistent inflammation which can progress to fibrosis. Emerging evidence suggests potential effects of NAFLD and NASH on the development of pathologies in the respiratory tract, but the interplay between the liver and the lung remains largely unexplored. In the current study, we assessed the impact of NASH on lung inflammation and fibrosis using a genetically modified mouse model lacking hepatic farnesoid X-receptor (FXR), a nuclear receptor involved in bile acid and lipid homeostasis, and lipocalin-2 (Lcn2), an acute phase protein upregulated in response to stress. Both FXR and Lcn2 are also involved in regulating innate immune responses. Wild type (WT), $Lcn2^{hep-/-}$, and $Lcn2/Fxr^{hep-/-}$ (DKO) mice were fed control (10% kCal) or high-fat (HF) (60% kCal) diets (n=5-10 mice/group). Liver, lung, serum, and bronchoalveolar lavage (BAL) fluid were collected after 1, 3, and 6 months of feeding. Histopathologic evaluation of livers from HFD-fed mice confirmed the development of NASH. In the lung, we observed histopathologic alterations including inflammatory cell infiltration, lipid-laden macrophages, septal damage, and epithelial thickening at 6 months; these alterations were most notable in HFD-fed DKO mice. Additionally, BAL cell counts were increased in HFD-fed DKO mice at 6 months when compared to HFD-fed WT mice, indicating lung inflammation and injury. Further analysis of expression levels of genes related to lung inflammation and lipid metabolism may reveal mechanisms underlying lung injury following the development of NASH. *Supported by NIH Grants ES029258, ES005022, and ES004738.*

1851 Exacerbation of Nanoparticle-Induced Pulmonary Inflammation in a Mouse Model of Metabolic Syndrome

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Engineered nanomaterials (ENMs) have the capacity to revolutionize numerous technologies however exposure-induced health effects are of concern. The majority of ENM safety evaluations have been performed utilizing healthy models. A growing proportion of individuals suffer diseases that may enhance their susceptibility to exposures. Specifically, metabolic syndrome (MetS) is increasingly prevalent and is a risk factor for the development of chronic diseases including type-2 diabetes, cardiovascular disease, and cancer. MetS is a combination of conditions which includes obesity, and the dyslipidemia. Due to the role of lipids in inflammatory signaling, we hypothesize that MetS-associated dyslipidemia may modulate ENM-induced immune responses. To address this hypothesis, mice were fed either a control diet or a high-fat diet for 14-weeks. A subset of mice were treated with atorvastatin for the final 7-weeks to modulate lipids. Mice were exposed to silver nanoparticles via oropharyngeal aspiration, and acute toxicity endpoints were evaluated 24-hours post-exposure. Mice on the high-fat diet demonstrated increased body weight, and cholesterol compared to control-diet mice. Cytometry analysis of bronchoalveolar lavage fluid demonstrated exacerbation of neutrophilic influx in MetS mice while statins reduced neutrophilia in MetS mice. Additionally, enhanced proinflammatory mRNA expression and protein levels of monocyte chemoattractant protein-1, macrophage inflammatory protein-2, and interleukin-6 were observed in MetS mice. These markers were reduced due to statin therapy. In contrast, ENM exposure reduced mRNA expression of enzymes involved in lipid metabolism, such as arachidonate 5-lipoxygenase, and arachidonate 15-lipoxygenase while statin treatment inhibited these alterations. A lipidomics approach was utilized to investigate lipid mediators of pulmonary inflammation. This assessment demonstrated reduced levels of lipids involved in inflammatory resolution (resolvins, maresins, EPA, DHA, etc.) in the MetS model compared to healthy following ENM exposure. Statin treatment inhibited these MetS-induced lipid alterations following ENM exposure. Taken together our data suggests that MetS exacerbates the acute toxicity induced by ENM exposure via a disruption of lipid mediators of inflammatory resolution leading to enhanced pulmonary inflammation.

1852 Anti-Inflammatory Role of Rosmarinic Acid against Cisplatin-Induced Acute Kidney Injury through Blockage of Caspase-1

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Cisplatin (CP) is one of the effective antineoplastic drugs used to treat solid tumors effectively. However, it induces acute kidney injury (AKI). Several signaling pathways are responsible to induce severe inflammation in renal proximal convoluted tubule cells leading to AKI. In this study, we investigated the therapeutic role of rosmarinic acid (RA) against the inflammation in AKI. RA has been demonstrated to have antioxidant, free radical scavenging and anti-inflammatory effects. In our study, RA was administered orally with the dose of 100mg/kg/d for consecutive four days after a single shot of cisplatin at the dose of 20mg/kg administered intraperitoneally in Swiss albino mice. We found that CP causes the oxidative stress by increasing the level of lipid peroxidation and decreasing the level of reduced glutathione (GSH) in kidney tissue, creating a favorable environment for AKI. CP exposure also increased the levels of serum creatinine and blood urea nitrogen (BUN) which indicates the alteration in the normal renal functions in the exposed mice. Inflammasomes are signaling complexes consisting of caspase-1 and nucleotide-binding oligomerization domain-like receptor with pyrin domain protein (NLRP). We found that inflammasome was activated by CP administration promoted the activation of caspase-1 and resultantly increased the secretion of IL-1β and IL18 in kidney tissue creating a proinflammatory environment. Treatment of RA inhibited the activity of the caspase-1 and other downstream molecules such as IL-1β and IL18, nuclear factor-kB and NLRP3. Also, elevated protein expression of COX-2 has been found to be down-regulated in RA treated mice. These findings show that blocking a critical step by RA in inflammation may be a useful strategy to prevent the AKI induced by cisplatin.

1853 Treatment with Cannabidiol Ameliorates Experimental MS by Regulating Expression of Gasdermins (GSDMs) and Regenerating Islet-Derived (Reg)-4 in the Intestinal Epithelial Cells

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Cannabidiol (CBD) is a recently legalized nonpsychoactive ingredient of Cannabis that has garnered attention in the media, medical community and general public due to its anti-inflammatory properties and therapeutic potential. CBD has become popular among individuals with autoimmune disorders due to its potential as complementary and alternative medicine, especially in patients with side effects associated with immunosuppressive regimens. There has been little work done on risk assessment and the mechanism of action of CBD. In order to better define how CBD may be beneficial, we studied the effects of oral administration of CBD (20mg/kg) in chronic progressive multiple sclerosis (MS) using a murine model of experimental autoimmune encephalomyelitis (EAE). We found that CBD significantly reduces the inflammation-related paralysis in EAE and that this was primarily due to the induction of myeloid derived suppressor cells (MDSCs). In CBD treated EAE mice, flow cytometric analysis revealed increase in the granulocytic MDSCs (CD45+CD11b+Ly6C+Ly6G+) and monocytic MDSCs (CD45+CD11b+ Ly6C+Ly6G-) in the CNS and spleen respectively. CBD treatment additionally led to significant decrease in the inflammatory neutrophils (CD45+CD11b+Ly6C-Ly6G-) in the mesenteric lymph nodes. In order to better understand the mechanism of action of CBD, we performed the transcriptomics of the intestinal epithelial cells (IECs). We found that the IECs from EAE mice treated with vehicle demonstrated several dysregulated genes, of which 3 of the most upregulated genes were Gsdmc3, Gsdmc3 and Gsdmc4 belonging to the Gasdermin (GSDM) family, and CBD treatment decreased their expression. GSDMs have been shown to drive inflammation through induction of pyroptosis of IECs and pyroptosis/NETosis of myeloid cells infiltrating the periphery. Another upregulated gene was regenerating islet-derived family member 4 (Reg4) in EAE+Veh group which was decreased in EAE+CBD. Reg4 has been shown to be a driving factor in inflammation by altering microbial composition and promoting the expression of pro-inflammatory cytokines. These results suggest a novel anti-inflammatory function of CBD in inflammatory disorders by altering gut-microbiota and preventing pyroptosis in the IECs by inhibition of GSDMs and Reg4 expression.

1854 Involvement of Galectin-3 in Maintaining Barrier Integrity of Lung Vasculature

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Galectin-3 (LGALS3; Gal-3) is a 35-kDa carbohydrate-binding protein used as a diagnostic biomarker for cardiovascular disease. Recently, a role for Gal-3 in the pathophysiology of heart failure and lung fibrosis has been suggested. Histological examination of lung sections from unchallenged Gal-3 -/- KO mice revealed elevated levels of perivascular inflammation relative to wild type mice. Normal lung vasculature typically exhibits a size selective permeability that permits small solutes to enter the airway but limits the influx of large plasma molecules. We hypothesized that Gal-3 -/- mice would also exhibit a disruption in airway vascular barrier function. To assess for "leakiness", we analyzed bronchoalveolar lavage for high molecular weight IgM (900 kDa) and performed immunohistochemical analysis to screen for the presence of plasma-derived fibrinogen and inflammatory cells in lung tissue. Both Gal-3 -/- and corresponding wild-type (WT) C57/bl6 mice were exposed to nitrogen mustard or phosphate buffered saline (PBS) by intratracheal instillation. Fourteen days after exposure, mice were terminally anesthetized and airway-capillary leak was estimated by the detection of IgM levels in bronchoalveolar lavage fluid (BALF). Left lung lobes from these mice were also paraffin embedded, sectioned at 5 µm thickness, and immunostained for the plasma protein fibrinogen. Deposition of fibrinogen in airways was assessed by use of an Allred scoring system. Additional sections were also immunostained for myeloperoxidase (MPO) to assess numbers of activated inflammatory cells in lung. In contrast to PBS-treated wild type mice, immunohistochemistry performed on Gal-3 -/- mice after PBS treatment, revealed prominent perivascular fibrin deposition, increased numbers of MPO-expressing inflammatory cells, and a four-fold elevation IgM plasma protein in BALF. GAL-3 may be an essential factor for maintaining hemostasis and barrier integrity of lung vasculature. *This work supported by Rutgers University CounterACT Center of Excellence - U54AR055073, and pilot grant funding from the Center for Environmental Exposures and Disease (CEED).*

1855 Modulation of TRP Channels by Lipids in Chronic Pain Models

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Chronic pain is pain lasting beyond the time required to heal an injury, which can severely affect a person's life. Chronic pain therapies include NSAIDs, opioids, and TNFα inhibitors. However, none are universally efficacious, or without risks. New therapeutic options are needed for chronic pain. Transient receptor potential (TRP) ion channels are integral to inflammation and pain following injury. Thus, understanding how endogenous TRP ligands change during inflammation and during the development of chronic pain may provide new insights on how to prevent or treat chronic pain. We are using transcripomics and targeted lipidomics to determine how changes in endogenous lipids affect TRP ion channels known to be involved in acute inflammation and in the development of chronic pain. RNA sequencing of mouse dorsal root ganglia (DRG) neurons following spinal nerve ligation (SNL) revealed an induction of cytochrome P450 1B1 and CYP4B1 in injured neurons. CYP1B1 and CYP4B1 produce hydroxyeicosatetraenoic acids (HETEs) which have pro-inflammatory properties. Moreover, screening of lipid extracts of spinal cord from sham and SNL groups demonstrated significant differences in the activation of TRPA1, M8 and V1. Notably, at day 14 post SNL, lipid extracts were less potent TRP agonists suggesting a down-regulation of TRP-mediated signaling following nerve injury. Collectively, these data suggest that alterations to TRP signaling in pain may involve a pool of endogenous TRP channel ligands that are synthesized and degraded in a dynamic manner. Targeted lipidomics studies are ongoing and should reveal specific endogenous lipids that modulate TRP channel activity over the course of development of chronic pain phenotypes. These results could advance our understanding of specific molecular processes that contribute to pain pathogenesis, as well as reveal new targets for potentially preventing and/or treating pain. *Support: DoD W81XWH-17-1-0413.*

1856 Beneficial Effects of the Immunomodulatory Glycan LNFPIII on the Microbiota in a Mouse Model of Gulf War Illness

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Gulf War Illness (GWI) is a chronic, multi-system disorder that presents in almost a third of the GW veterans. Stemming from exposures to pesticides, prophylactics and, in some cases, nerve agents, GWI affects the nervous, immune, and gastrointestinal systems, potentially dysregulating the gut-brain-immune axis. Developments in our understanding of the microbiota's influence on host physiology have sparked interest in the role the microbiota might play in GWI. By rebalancing the immune system, the immunomodulatory glycan Lacto-N-fucopentaose III (LNFPIII), might be beneficial to GWI veterans. Given the crosstalk between the microbiota and the immune system, this study sought to understand whether LNFPIII treatment is beneficial to the gut microbiota in a GWI context. We examined the short- and long-term effects of GWI chemical exposures using an established GWI model (PB/PM). Male C57BL/6 mice were treated with the neuro-prophylactic pyridostigmine bromide (PB; 0.7 mg/kg IP) and the insecticide permethrin (PM; 200 mg/kg) for 10 days. In the short-term study, LNFPIII (35 ug; SC) or dextran vehicle was given concurrent with GWI chemicals. In the long-term study, LNFPIII treatment (twice/week) started 4 months after PB/PM treatment ended and it was given until study completion. Fecal microbiota analysis (16S rRNA sequencing) was performed 6 and 48 h (short-term study) or 9 months (long-term study) post PB/PM exposure. GWI chemicals affected the global microbiota profile in both the short and long-term. Limited specific effects were observed in the short-term, but the genus *Sutterella* was increased by PB/PM in both short and long-term samples. PB/PM had multiple long-term effects, with most significantly affected OTUs aligning to *Lachnospiraceae* and *Ruminococcaceae* families. LNFPIII treatment increased the relative abundance of butyrate producing bacteria (e.g., *Butyricoccus*, *Ruminococcous*) in PB/PM-treated mice, indicating positive selection pressure for these potentially beneficial bacteria. Additionally, two *Ruminococcaceae Oscillospira,* four *Lachnospiraceae*, one *Rikenellaceae*, and one *S24-7* OTUs correlated in opposite directions with lipocalin II (LCN2), a marker of intestinal inflammation in PB/PM vs PB/PM/LNFPIII samples. Overall, we found that the most significant GWI effects on the microbiota occurred in long-term studies and that LNFPIII promotes microbiota changes that could be explored as a GWI therapeutic. *Support: Department of Defense grant number W81XWH-16-1-0586.*

1857 A Novel Macrophage Containing Organotypic Small Intestinal Tissue for Modeling Gut Inflammation

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As gate keepers of intestinal immune homeostasis, macrophages play a critical role in inflammation in the gut. In this study, we reconstructed a new macrophage-containing primary cell-based full-thickness small intestinal (SMI+M) tissue model and characterized it for 1) macrophage incorporation (immunohistochemistry, IHC), 2) barrier properties (TEER), and 3) functionality by measuring inflammatory responses following exposure to ligands for TLR4 (lipopolysaccharide; LPS), NOD-1 (C12-iE-DAP) and NOD-2 (L18-MDP) either individually or synergistically. For identification of inflammatory responses, we utilized Affymetrix GeneChip arrays. Results showed that SMI+M tissues have 3D polarity and their morphology and physiological barrier property mimics that of native *in vivo* tissues. IHC of SMI+M tissues showed CD14+ (macrophage marker) cells in the underlying fibroblast layer. Using gene upregulation level of >1.9 fold as a cut-off following ligand stimulation, tissues without macrophages (SMI-M) showed fewer upregulated genes (1400 genes) compared to SMI+M (4400 genes). Furthermore, when gene upregulation levels by ligand-induced SMI+M were compared against stimulated SMI-M even higher differences in upregulated genes (> 5200 genes) were noted. In SMI+M, upregulated genes include chemokines, chemokine receptors, FC receptors, co-stimulatory molecules, interferons, and HLA's which are characteristic of immune cells. When we looked at 13 upregulated genes (>6-fold) in SMI+M, the synergistic effect of ligands in inducing inflammatory gene upregulation was much more pronounced compared to stimulated SMI-M tissues. Furthermore, increased cytokine release of IL-6, IL-8, and TNF-α into the culture medium from ligand exposed SMI+M was also confirmed by BioPlex ELISA. In summary, our results demonstrate that the 3D SMI+M tissue model can serve as an *in vitro* tool to study the complex cellular interactions manifested during inflammation in the gut microenvironment.

1858 The Effect of Circadian Disruption on Hepatic Inflammation and Cancer Formation

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Incidence of hepatocellular carcinoma (HCC) has steadily increased over the last several decades and has proven to be one of the least responsive to current therapeutic options. Recently, circadian disruption has been shown to induce non-alcoholic steatohepatitis that often progresses to HCC. Circadian rhythms are normal biologic processes that coordinate cell and organ function with a 24-hour period. The disruption of these rhythms has been shown to promote inflammation and cancer development, but the effect on HCC development is largely unknown. To investigate a potential pathway for development of liver inflammation and HCC, we began by evaluating C57BL6/J (WT) mice under normal circadian (WT_N) and circadian disrupted (WT_D) conditions and compared transcriptomic profiles between the groups. A total of 144 mice were divided into 2 age-matched, cage-matched groups comprised of equal representation of male and female mice. At 4 weeks of age, the WT_N group was kept on a standard 12:12, light-dark cycle to establish a "normal" circadian rhythm and the WT_D group began a jet-lag protocol consisting of a 4-hour time shift every 2 days for 4 weeks to induce circadian disruption. At 8 weeks of age, 6 WT_N and 6 WT_D of equal males and females were sacrificed every 4 hours over a 48-hours window. Hepatic mRNA was immediately isolated from the 12 time-points in order to illustrate gene expression throughout the day. Core Clock Genes (CCGs) were analyzed with real time quantitative polymerase chain reaction (RT-qPCR). We found that the jet-lag protocol did not have a major impact on the relative expression of Nuclear Receptor subfamily 1 group D member 1 (NR1D1) and Cryptochrome Circadian Regulator 2 (*Cry2*). Opposingly, Cryptochrome Circadian Regulator 1 (*Cry1*) and Nuclear Factor Interleukin 3 (*Nfil3*) were significantly disrupted experiencing rhythm loss and phase shift respectively. The time point 22:00 was identified as the most sensitive time point with significant fold change and max peak heights for both *Cry1* and *Nfil3*. This study identifies CCGs and select inflammatory regulators were disrupted in the liver. This information is crucial to establish a baseline for circadian disruption in the liver, guiding future studies into the development of HCC in inducible transgenic mouse models.

1859 Ampakine CX546 Ameliorates Gulf War Agents Exposure and Stress-Induced Exosomal HMGB1 That Causes Neurological Ailment in Experimental Gulf War Illness

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Gulf War Illness (GWI) is a medically unexplained, multisymptomatic condition that includes chronic pain, gastrointestinal and neuroinflammation, and cognitive difficulties. Exposure to Gulf War (GW) agents pyridostigmine bromide (PB) and permethrin (Per), and war theater stress were key contributors to the etiology of GWI post-deployment to the Persian GW. In this study, we examined the role of circulatory exosomal high mobility group box-1 (HMGB1) protein in neuroinflammation, neuroimmunotoxicity, and neuroplasticity in the mouse model of GWI. Here, we used an established mouse model of GWI. The C57BL/6 mice were exposed to GW agents (PB+Per) along with restraint stress for one week. Results show that GW agent exposure along with stress causes exosomal biogenesis and HMGB1 expression in the small intestine and concurrently increase circulatory exosome loaded with HMGB1. Data also show decrease Zonal Occludin-1 (ZO1) and Claudin-1 mRNA expression, and serum albumin accumulation in frontal cortex suggests blood-brain barrier (BBB) integrity loss that enables entry of circulatory exosomal HMGB1 in the brain. Increase HMGB1 in both the frontal cortex and hippocampus activate microglia (M1 phenotype), thus induces an inflammatory response. We observed that HMGB1 induced neuroinflammation suppress brain-derived neurotropic factor (BDNF) and thus causing neuroplastic and cognitive impairment. The GW agents exposed mice co-treated with exosome inhibitor (Nexinhib20), or glutamate activator (ampakine, CX546) show improvement in BBB integrity, neuroinflammation, neuroplasticity, and cognitive function. In summary, our findings suggest that the circulatory exosomal HMGB1 plays a key role in GWI pathogenesis, and Nexinhib 20 and CX546 might be promising therapeutics in GWI. *This study was supported by W81XWH1810374 and VA merit award I01 CX001923-01 to Saurabh Chatterjee.*

1860 The Effects of Age, Sex, and Genotype on LPS-Induced Neuroinflammation in Humanized Targeted Replacement APOE Mice

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Neuroinflammation is implicated in the progression and pathogenesis of several neurodegenerative diseases including Alzheimer's disease (AD). While AD presents differently in individual patients, advancing age and the presence of the strongest known genetic risk factor, Apolipoprotein E4 genotype, have been shown to contribute greatly to the increased risk of AD. In addition, females have an increased risk of developing AD at a younger age when compared to males and this risk is modified by the Apolipoprotein E (APOE) genotype. Here, we sought to determine age, sex and APOE genotype susceptibility to neuroinflammation following administration of lipopolysaccharide (LPS; 0.5 mg/kg for 4 hours) in humanized targeted replacement APOE3 and APOE4 mice. Using quantitative PCR, we evaluated the proinflammatory cytokines Il1b and Tnfa in the cortex and hippocampus. LPS caused a higher induction of pro-inflammatory cytokines, Il1b and Tnfa mRNA expression in both the frontal cortex and hippocampus of young (3-month-old) and aged (16-month-old) APOE4 mice compared to APOE3. Il1b mRNA levels were increased in the frontal cortex by ~30-fold in aged APOE4 males and ~17-fold in APOE4 females. In contrast, Il1b expression only increased ~5-fold and ~7-fold in APOE3 males and females, respectively. In the hippocampus, there were no differences by genotype in the young mice, but aged APOE4 males and females exhibited a higher Il1b response than the young mice. Similar effects were observed for Tnfa expression in both regions, with differences apparent at 16 months for both APOE4 males (~9-10-fold increase in both, the frontal cortex and hippocampus) and females (~10-fold in both, the frontal cortex and hippocampus). These data indicate that a peripheral LPS challenge induces a higher increase in pro-inflammatory cytokine mRNA expression in older APOE4 targeted replacement mice compared to APOE3 and this effect appears to be sex-specific at different age groups. *Supported in part by NIH R01ES026057.*

1861 Redox-Associated Modulation of O-GlcNAcylation Signaling Ameliorates Liver Injury Induced by Co-exposure to Ethanol and Lipopolysaccharide in Mice

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Both alcohol consumption and endotoxin [lipopolysaccharide (LPS)] exposure can induce oxidative liver injury, the synergetic effect of which is hypothesized to contribute significantly to the deleterious progression of alcoholic liver disease (ALD). O-GlcNAcylation of protein is an emerging form of post-translational modification, where a single O-linked N-acetylglucosamine moiety is added to Ser and Thr residues of nuclear, cytoplasmic and mitochondrial proteins. This process is controlled by a pair of enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). O-GlcNAcylation of protein is increasingly recognized as playing an important role in stress sensing and fine-tuning of inflammatory response. To date, little is known about the role of O-GlcNAcylation in ALD pathogenesis. In the current study, we investigated the impact of binge alcohol drinking in combination with LPS exposure on hepatic O-GlcNAcylation pathway in a mouse model of chronic glutathione (GSH) deficiency. Female wild-type (WT) and GSH-deficient *Gclm*-null (KO) mice received ethanol for 3 consecutive days (5 g/kg i.g. per day), followed by a single LPS administration (10 mg/kg i.p.). At 0, 6,12 and 24 hr post LPS treatment, liver injury, expressions of inflammatory genes and the O-GlcNAcylation pathway in the liver were examined. Compared to WT mice, KO mice had a lower injury score at 24 hr post LPS treatment. The expression profile of inflammatory genes and O-GlcNAcylation-related genes revealed differential changes between WT and KO livers at 12 and 24 hr post LPS treatment. There was an overall increase of O-GlcNAcylation of liver proteins in KO mice by ethanol binge feeding alone and by ethanol-LPS co-treatment at 24 hr. In conclusion, GSH-deficient mice display a partial protection against liver injuries caused by acute ethanol-LPS co-exposure. We speculate that modulation of O-GlcNAcylation signaling by low GSH may serve as a protective mechanism. *This work was supported, in part, by NIH grants K01AA025093, P30DK034989, R24AA022057 and International Communication of Guangxi Medical University Graduate Education.*

1862 Microplastics Pose a Serious Risk to the Gut Immune System

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Plastic pollution has increased exponentially for the past 70 years; in 2015 we passed a watershed mark of *1 metric tons of plastic per person on earth*. Slow breakdown of plastic materials primarily leads to smaller and smaller microplastic particles (MP), but not actual degradation or decomposition of the material. We now face extensive microplastic contamination of our food and water sources, raising ecological and public health concerns. MP ingestion by humans is now an inevitable consequence of global plastic pollution, and we are only now becoming aware of this burgeoning crisis. Currently, there is inadequate research available to fully comprehend how MP impact human health (WHO). There is also an important gap in knowledge regarding how MP affect the major direct organ of contact, the gastrointestinal (GI) tract. We provide compelling evidence that MP affect both intestinal epithelium and macrophages. We show: a) MP increase intestinal permeability in human intestinal organoid monolayer cultures as well as impede wound healing in Caco-2 monolayers; b) MP are engulfed by macrophages which leads to an activated state; c) MP engulfment by macrophages induces a metabolic shift towards glycolysis, an immunometabolic active state; d) autophagy, a cellular degradative catabolic process, can dampen the glycolytic shift in macrophages after MP engulfment; and e) MP ingestion affects the weight of mice and this weight modulation is independent of intestinal epithelial TLR4 expression. In summary, our findings indicate MP ingestion poses a serious human health hazard by disrupting oxidative metabolism in both intestinal epithelial cells and macrophages subsequently causing intestinal permeability, dysbiosis, and an immunometabolic active state which could lead to intestinal and systemic inflammation.

1863 Homogeneous, Bioluminescent Cytokine Assay Applied to a Monocyte Activation Test for Pyrogen Detection

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Pyrogens cause cytokine release from cells such as monocytes as a protective response to infection and injury. However, an exaggerated pyrogen response can cause systemic inflammation, shock, organ failure and death. Injectables and implanted devices are tested for pyrogens, especially endotoxins from gram-negative bacteria, because they can be left as residuals after manufacturing and disinfection processes. Non-endotoxin pyrogens are also a concern, and increasingly so with the expanded use and complexity of biologic drug formulations that may carry pyrogen contamination. Standard pyrogen tests include the endotoxin-selective, limulus amebocyte lysate (LAL) test and the broad-spectrum rabbit pyrogen test (RPT). Monocyte Activation Tests (MAT) were recently approved as an alternative pyrogen test to detect endotoxin and non-endotoxin pyrogens and avoid animal use. MATs measure IL-1β or IL-6 cytokines released from monocytes by ELISA. To simplify the MAT workflow, we developed a homogeneous cytokine assay method without transfer or wash steps to replace the ELISA. For a given target, two selective antibodies are respectively labeled with complementary subunits of Nanoluc® luciferase. When labeled antibody pairs bind their target, the subunits are brought into proximity to reconstitute a bright luciferase that generates light with the substrate furimazine. Appropriate antibody pairs for either IL-1β or IL-6 were selected and applied to 4-donor pools of peripheral blood mononuclear cells (PBMCs). Release of IL-6 or IL-1β from PBMCs was detected after treatment with several pyrogens, including LPS, R848, lipoteichoic acid, Pam3CSK4, and heat-killed *Staphylococcus aureus*. Both assays are sensitive with limits of detection of ~10 pg/ml for IL-1β and ~5 pg/ml for IL-6 and have large linear dynamic ranges of greater than 3 logs. However, the IL-6 assay had greater sensitivity for all tested pyrogens and a larger response (3-15 fold greater, depending on pyrogen). Bacterial flagellin, another pyrogen, was only detected in the IL-6 immunoassay. The IL-6 immunoassay had a limit of detection of ~0.03 endotoxin units/ml. This homogeneous IL-6 assay enables a sensitive MAT in an add-and-read format that reduces ELISA workflow steps and facilitates assay automation. This simplified MAT is an attractive RPT alternative that detects a broad spectrum or pyrogens.

1864 Cytochrome P450 (CYP) 2C8*3 Alters Lipid Biosynthesis and Inflammatory Responses of Human Lung Epithelial Cells

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Asthma causes chronic airway inflammation and bronchial hyper-reactivity. Despite appropriate treatment, many patients experience suboptimal symptom control, in part, due to variability in genes that dictate drug disposition. A total of 170 single SNPs in genes associated with asthma, as well as variants of unknown significance, were assayed for effects on asthma control in a pediatric asthma cohort. The presence of one or more copy of the cytochrome P450 (CYP) 2C8*3 (R139K/K399R) allele was found to correlate with improved asthma control: Mean asthma control scores were 3.5 [n=184] vs. 4.4 [n=909] for the CYP2C8*1/*1 genotype. Furthermore, when data were stratified by treatment with montelukast, patients with the CYP2C8*3 allele exhibited a mean asthma control score of 3.56 [n=55] vs. 5.44 [n=214] for the CYP2C8*1/*1 genotype (p=0.0017). This effect was not observed for several other asthma medications. CYP2C8 is the principal enzyme involved the clearance of montelukast, a cysteinyl leukotriene receptor (CysLTR) antagonist prescribed for moderate to severe asthma. Using human lung epithelial cells engineered to over-express equivalent levels of either CYP2C8*1 or *3, it was found that CYP2C8*3 exhibits greater intrinsic activity towards montelukast, suggesting that increased or prolonged inhibition of the CysLTR due to decreased clearance of montelukast was likely not a basis for CYP2C8 genotype-dependent differences in asthma symptom control. CYP2C8 also metabolizes endogenous long-chain polyunsaturated fatty acids. Results from lipid profiling show differences in the production of specific lipid mediators of inflammation by human lung epithelial cells, and effects on epithelial wound repair *in vitro*. Further, expression of CYP2C8*3 alters mRNA abundance of several additional mediators of inflammation with relevance to asthma pathogenesis. These, and future findings should further our long-term goal of improving treatment of asthma through better understanding of the mechanisms associated with sub-optimal responses to current therapies. *Support: GM121648.*

1865 The Inflammatory Role of the Infrapatellar Fat Pad in a Model of Spontaneous Osteoarthritis

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Osteoarthritis (OA), particularly knee OA, is a painful and debilitating disease affecting millions of people worldwide. Currently, there are no therapeutic regimens that halt or repair this joint degeneration. The knee is a unique joint due to the large amount of adipose present, which serves as a rich source of lipids. Lipids provide a diverse range of physiologic functions, including energy storage, endocrine signaling, and structural components of cells. However, lipid-mediated signaling also drives a plethora of pathologic processes. Despite the infrapatellar fat pad (IFP) being the largest adipose deposit in the knee, its role in joint homeostasis is not clearly defined. It is hypothesized to provide lubrication, shock absorption, and stability, but may also serve as a source of inflammation. It is similar to white adipose tissue and contains a number of resident and circulating immune cells. We recently completed transcript expression analysis of the IFP in the Hartley guinea pig model of non-traumatic/primary OA at 3 key ages. We demonstrated that peroxisome proliferator-activated receptor gamma (PPARgamma) and adiponectin decreased in expression throughout aging, while tumor necrosis factor (TNF) and interleukin - 1 beta (IL-1B) increased. Thus, the IFP may become a lipotoxic adipose depot with age. To assess the role of the IFP in OA, we conducted a study to determine the influence of IFP removal on disease progression. We postulate that, if the IFP is lipotoxic, removal would be beneficial to OA. Prior to OA-onset, 3-month-old guinea pigs underwent surgical removal of the IFP from one knee (sx) while the contralateral knee received a sham surgery. Animals were harvested at 7 months of age. Histologic analysis of the sx knee indicated the development of fibrous connective tissue (FCT) in place of the removed IFP. Relative to the knee containing the IFP, the FCT was decreased in OA-associated structural changes to both the cartilage and bone. Further, protein expression of the FCT versus IFP confirmed a marked decrease in monocyte chemoattractant protein-1 (MCP-1) expression. This data suggests that the IFP plays an inflammatory role in the pathogenesis of OA in Hartley guinea pigs and that removal prior to disease onset appears to have short-term benefits on joint health.

1866 Altered Gut Virome-Bacteriome Diversity Regulates Inflammatory Phenotype and Neuronal Immunotoxicity in Experimental Gulf War Illness

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Gulf War illness (GWI) is characterized by the persistence of inflammatory bowel disease, chronic fatigue, neuroinflammation, headache, cognitive impairment, and other medically unexplained conditions. Results using a murine model show that enteric viral populations especially bacteriophages were altered in GWI. The increased viral richness and alpha-diversity correlated positively with gut bacterial dysbiosis and proinflammatory cytokines. Altered virome signature in GWI mice also had a concomitant weakening of intestinal epithelial tight junctions with a significant increase in Claudin-2 protein expression and decrease in ZO1 and Occludin mRNA expression. The altered virome signature in GWI, decreased tight junction protein level was followed by the presence an activation of innate immune responses such as increased Toll-like receptor (TLR) signaling pathways. The altered virome diversity had a positive correlation with serum IL-6, IL-1β, and IFN-γ, intestinal inflammation (IFN-γ), and decreased Brain-Derived Neurotrophic Factor (BDNF), a neurogenesis marker. The co-exposure of Gulf War chemical and antibiotic (for gut sterility) or Gulf War chemical and Ribavirin, an antiviral compound to suppress virus alteration in the gut showed significant improvement in epithelial tight junction protein, decreased intestinal-, systemic-, and neuroinflammation. These results showed that the observed enteric viral dysbiosis could activate enteric viral particle-induced innate immune response in GWI and could be a novel therapeutic target in GWI. *This study was supported by W81XWH1810374 and VA merit award I01 CX001923-01 to Saurabh Chatterjee.*

1867 Mechanisms of Ectopic TRPA1 Expression by Human Lung Epithelial Cells with the TRPV1 I585I/V Genotype

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Asthma is a chronic inflammatory disease that is often exacerbated by environmental pollutants. Transient receptor potential ankyrin-1 (TRPA1) and vanilloid-1 (TRPV1) are activated by multiple environmental pollutants including coal fly ash and diesel exhaust particles. Studies have examined roles for TRPA1 and V1 in asthma and have shown that the expression of these channels is elevated in epithelial cells and neurons in people with asthma. Our previous studies demonstrated that the I585I/V genotype of TRPV1 affected asthma symptom control. Moreover, the TRPV1 I585I/V genotype was associated with increased TRPA1 expression and sensitivity of primary human lung epithelial cells to diesel exhaust particles. Specifically, increases in the expression of mRNA for IL-8 and DNA damage-inducible transcript-3 (DDIT3), a pro-apoptotic ER stress response gene, were greater among donor cells with the I585I/V genotype (n=6) versus wild-type (I585I/I) donors (n=7). Cytokines like IL-8 regulate key facets of pulmonary inflammation, and ER stress plays a fundamental role in the pathogenesis of inflammatory diseases, such as asthma. Cells with the I585I/V genotype also show differential expression of NF-κB regulatory factors including NLRP2, which is a negative feedback inhibitor of the NF-κB/HIF-1α signaling pathway and potential regulator of TRPA1 expression during inflammation. Studies are ongoing to understand how TRPA1 expression is influenced by the I585I/V genotype, and elucidating this relationship will advance our understanding of a phenomenon that may represent a risk factor for asthma pathogenesis. *Support: ES017431, ES027015, and GM 121648.*

1868 Cell and Molecular Biology of Autoimmunity Affecting Behavior of Mice with Deviant Behaviors

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Female and male mice of the BTBR *T*$^{+}$ *Itpr3*^{tf}/J (BTBR) strain have behaviors that resemble autism spectrum disorder. BTBR mice have better humoral immunity than cell-mediated immunity, in that they have naturally high serum IgG levels and generate high levels of IgG antibodies, including antibodies to brain antigens, but they have poor host defense against *Listeria*. Focus is on induction of an autoimmune-like phenotype. BTBR IgG autoantibodies bind to neurons better than microglia and with highest titer to nuclear antigens. BTBR thymi have more single-positive T cells and fewer double-positive T cells than C57BL/6/J (B6) mice. In blood and spleen, BTBR mice have more T cells (CD4^{+} and CD8^{+}) than B6 mice, but they have fewer B cells and NK cells. Since BTBR have an increased number of T-bet^{+} and GATA-3^{+} T cells their low defense against *Listeria* may be due to fewer NK cells. Their higher levels of autoantibodies is likely due to more splenic T follicular helper (Tfh) cells and plasma cells (PCs), which are higher in BTBR than B6 mice. Increased B cell differentiation to PCs may relate to the increased splenic mRNA expression of Notch1, 2 and 3, Jag1, Dll1/4, Hey1 and 2, and IL-21R. Less anti-oxidant response capacity measured as low glutathione and leukocyte surface thiol levels may be responsible for the BTBR's immunity. An elevated level of stress like stress-induced sympathetic nervous system skewing of immunity is suggested to be responsible for the oxidative changes causing the altered immunophenotype and the autoimmune problems.

1868a Elevated Expression of Inflammatory Pathways Resulting from Dysregulated WNT Signaling Prior to Trauma Exposure Is a Predictor of Development of PTSD

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Posttraumatic stress disorder (PTSD) used to be considered a psychiatric disorder until a few years ago when reports showed that PTSD patients have dysregulated immune functions and experience chronic inflammation. It is a disorder which develops after a traumatic experience but the molecular mechanism of the disease pathophysiology is still not clear. It is very interesting to note that only some of the trauma-exposed individuals develop the disorder. To understand this biased mechanism, we analyzed blood transcriptome data generated from individuals before trauma exposure and compared with those who did not develop PTSD even after exposure to trauma. We observed that interferon signaling pathway was significantly dysregulated. In particular, Th1 signaling pathway genes, including STAT1, STAT2 and JAK1 were significantly upregulated in PTSD patients prior to trauma exposure. We also observed that genes downstream of JAK/STAT signaling were significantly upregulated. In the past, we have shown that increased WNT signaling activity was evident in PBMCs collected after PTSD development. Moreover, we showed that increased WNT signaling can drive elevated expression of IFNG and IL17 in pre-activated PBMCs. In line with this, in the present study, we observed that Axin1 (WNT signaling inhibitor) was significantly down regulated even before trauma exposure indicating that WNT signaling pathway could be dysregulated even before trauma exposure in individuals that develop PTSD. Altering the levels of Axin1 either by siRNA based knockdown or employing AXIN1 stabilizers as WNT signaling inhibitor provided further evidence that downregulation of AXIN1 could strongly favor the elevated expression of inflammatory genes. These results collectively imply that individuals that develop PTSD have an elevated activity of proinflammatory signaling pathway including the JAK/STAT mediated Th1 pathway even before exposure to trauma. Our data also indicates that increased activity of WNT signaling drives the elevated expression of Th1 pathway genes possibly leading to inflammatory state in these patients. Therefore, we conclude that elevated pro-inflammatory biomarkers could probably predict those individuals who are predisposed to developing PTSD. *Supported by NIH grants P01AT003961, P20GM103641, R01AT006888, R01ES030144, R01AI129788 and R01AI123947.*

1869 Interplay of Oxidative Stress, Toll-Like Receptor, and Nrf2 in Trichloroethene-Mediated Autoimmunity

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Oxidative stress is involved in trichloroethene (TCE)-mediated autoimmunity, as evident from our earlier studies in MRL+/+ mice. However, molecular mechanisms underlying the autoimmunity remain to be fully elucidated. Even though Toll-like receptor (TLR) signaling and nuclear factor erythroid-derived 2-like 2 (Nrf2) pathway have been implicated in autoimmune diseases (ADs), the role and interplay of oxidative stress, TLR and Nrf2 in TCE-mediated autoimmune response remain unexplored. This study was, therefore, aimed to better understand the role of oxidative stress in TCE-induced autoimmunity and its link with TLR and Nrf2. Groups of female MRL+/+ mice were treated with TCE, sulforaphane (SFN, an antioxidant) or TCE+SFN for 6 weeks (TCE, 10 mmol/kg, i.p., every 4th day; SFN, 8 mg/kg, i.p., every other day). TCE exposure led to significant increases in serum antinuclear antibodies (ANA) along with greater formation of hydroxynonenal (HNE)-specific circulating immune complexes as well as enhanced protein oxidation (carbonylation), suggesting an association of increased oxidative stress with autoimmune

response. Moreover, TCE exposure also resulted in increased expression of TLR4, Myeloid differentiation primary response 88 (MyD88), Interleukin-1 receptor-associated kinase-like 4 (IRAK4), NF-kB and reduced expression of Nrf2 and HO-1 in the spleen. Remarkably, SFN supplementation not only attenuated the TCE-induced oxidative stress, upregulation in TLR4 and NF-kB signaling, as well as downregulation of Nrf2, but also the autoimmunity marker ANA. These results, in addition to providing further support to a role of oxidative stress, also suggest that an interplay among oxidative stress, TLR4 signaling and Nrf2 pathway contribute to TCE-mediated autoimmune response. Furthermore, attenuation of TCE-induced autoimmunity by SFN provides an avenue for preventive and/or therapeutic strategies for ADs. *Supported by NIH ES016302 and ES026887.*

1870 Dysregulation of Nrf2 Signaling Contributes to Trichloroethene-Mediated Autoimmunity: Protective Role of Sulforaphane

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Trichloroethene (TCE), an occupational and ubiquitous environmental contaminant is associated with the induction of autoimmune diseases (ADs). Although oxidative stress plays a major role in TCE-mediated autoimmunity, the underlying molecular mechanisms still need to be delineated. Nuclear factor (erythroid-derived 2)-like2 (Nrf2) is an oxidative stress-responsive transcription factor and provides protection by regulating cytoprotective genes. However, the potential of Nrf2 in the regulation of TCE-mediated autoimmunity is not known. This study was, therefore, focused on establishing: (1) status and contribution of Nrf2 in TCE-mediated inflammatory and autoimmune responses during disease progression, and (2) protective role of antioxidant sulforaphane against trichloroethene-mediated autoimmunity. Female MRL+/+ mice were treated with TCE (0.5 mg/ml) for 24, 36 and 52 wks. Evaluation of samples from these mice showed that TCE exposure led to reduction in Nrf2 and HO-1, but significantly increased phos-NF-κB (p65) and iNOS along with increased p38 MAP kinase and pJNK expression in the liver. TCE exposure also increased hepatic protein carbonyl expression, further supporting the role of oxidative stress in TCE-mediated autoimmunity. To explore the role of Nrf2 in TCE-mediated autoimmunity, we treated female MRL+/+ mice with TCE (10 mmol/kg) along with/without antioxidant sulforaphane (8 mg/kg) for 6 wks. Interestingly, sulforaphane treatment led to Nrf2 activation and amelioration of TCE-mediated effects, as evident from reduction in inflammatory and autoimmune responses such as phos-NF-κB (p65) and iNOS along with suppression of anti-dsDNA antibodies. Furthermore, we observed that TCE treatment modulated inflammatory miRNA expression, including miRNA-690, miRNA-125a-5p and miRNA-21, which potentially target NFkB, and the changes in these miRNAs were attenuated by sulforaphane treatment. Our results show that TCE mediates an impairment in Nrf2 regulation. Attenuation of TCE-mediated autoimmunity via activation of Nrf2 supports that antioxidant sulforaphane could be a potential therapeutic candidate for autoimmune diseases. *Supported by NIH RO1 grants ES026887 and ES016302.*

1871 Protein Allergenicity Assessment Using Proteomic and Bioinformatic (AllerCatPro) Analyses

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Proteins in natural substances used in consumer products may present a risk for IgE-mediated respiratory allergies, especially when used in spray products. If no data are available to prove otherwise, 100% of the total protein content is considered allergenic. Here, we describe a novel approach to refine the risk assessment, exemplified with a theoretical consumer spray, an occupational exposure benchmark and exposure assessment using ConsExpo. Proteins are identified and semi-quantified by label-free proteomics analysis: normalized amount of protein was suspended (4% Sodium dodecyl sulfate, 100 mM dithiothreitol, 100 mM Tris, pH 7.6) and digested. Eluted peptides were introduced to a QExactive HF mass spectrometer (Thermo). Identified sequences were matched with UniProt database and quantified as sum of fragment ion intensities for all spectra counted for a protein, normalized by length and number of unique peptides. Allergenic potential is predicted based on FASTA sequences and AllerCatPro (allercatpro.bii.a-star.edu.sg), which predicts the allergenic potential of proteins based on comparison of 3D structure and amino acid sequence to a dataset of 4180 unique sequences derived from the union of the major databases FARRP, COMPARE, WHO/IUIS, UniProtKB and Allergome. The corn-derived material contains 0.22% total protein, 2009 unique proteins and the most abundant proteins comprise each 9.7% of total protein content. Exposure assessment with 10% protein content (0.055 ng/m^3/day) compared to the benchmark (1 ng/m^3/day) results in a Margin of Safety of 18, which is sufficient to protect from IgE-mediated respiratory allergies. The new information generated on the protein content and potential for allergenicity of natural substances can be used to refine the IgE-mediated respiratory allergy safety assessment and opens new opportunities for IgE-mediated allergy research of protein-containing materials.

1872 Classification of Human Reference Data for Skin Sensitization

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To support the evaluation of non-animal approaches for skin sensitization assessment, we collected data for over 2500 human predictive patch tests (human maximization test and human repeated insult patch test) from more than 1500 publications. Each test was evaluated for reliability. Results from 1900 tests considered to be sufficiently reliable were classified using the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Human predictive patch test data based on single doses makes identification of thresholds uncertain and difficult to use as reference data. To overcome this challenge, classification criteria from the GHS were extended using a decision tree to partly resolve ambiguity in the results. If individual chemicals had multiple discordant test results, a weight-of-evidence approach was used to arrive at a single classification for each chemical. This classification approach was applied to a Cosmetics Europe reference list of 128 substances to support the evaluation of defined approaches for skin sensitization proposed for inclusion in a new OECD guideline. While 79 substances could be classified, the data for 49 substances were not sufficiently reliable. Classifications for the 79 substances included: eight strong sensitizers (1A), four strong sensitizers with some likelihood of over-classification (1A-), one moderate sensitizer with some likelihood of under-classification (1B+), 28 moderate sensitizers (1B), 10 non-sensitizers with some likelihood of under-classification (NC+), and six non-sensitizers (NC). Twenty-two substances could not be classified as sensitizers or non-sensitizers, but a potential for strong sensitization could be ruled out (NC/1B). The entire human skin sensitization patch test database will be made publicly available in the future for additional evaluation of alternative skin sensitization methods and development of new models. *This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. The views expressed above do not necessarily represent the official positions of any federal agency.*

1873 Development of a Screening Assay System to Predict the Intrinsic Immunogenicity of a Drug or Compound

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Drug hypersensitivity is an unexpected and difficult to predict adverse event that occurs during clinical trials or clinical use. *In vitro* T-cell priming assays that assess the intrinsic immunogenicity of drugs have been developed; however, their application domain is limited due requirements for (i) large blood volumes, (ii) two batches of autologous dendritic cells, and (iii) an experienced researcher. Hence, we aimed to develop a simplified assay, termed the T-cell multiple well assay (T-MWA) that permits assessment of whether drugs activate T-cells, alongside analysis of the strength of the induced response and the number of cultures that respond. Dendritic cell T-cell priming was conducted in multiple wells of a 96-well U-bottomed plate, for two weeks with model haptens (nitroso sulfamethoxazole (SMX-NO), Bandrowski's base (BB) or piperacillin (PIP)). Cultures were then re-challenged with hapten and T-cell proliferation was measured using [3H]-thymidine incorporation. Priming of naïve T-cells was observed with SMX-NO in all experiments, with no requirement for dendritic cells during restimulation. Greater than 65% of cultures were activated with SMX-NO; 8.0, 30.8 and 27.2% of cultures displayed weak (stimulation index SI=1.5-1.9), good (SI=2-3.9) and strong responses (SI greater than 4), respectively. The number of responding cultures and strength of the induced response was reproducible when separate blood

donations from the same donors were compared. Checkpoint blockade with anti-PD-L1 and anti-CTLA4 antibodies increased the strength of the proliferative response against SMX-NO, but not the number of responding cultures. Good to strong priming responses were detected with BB, while PIP stimulated only a small number of cultures to proliferate weakly. Inducible CD4+CD25+FoxP3+CD127LOW Tregs were generated during priming to the haptenic compounds. In conclusion, this new form of priming assay offers improvements over existing assays. Furthermore, with development it could be used to study multiple HLA-typed donors in a single plate format.

PS 1874 Generation and Characterization of Clozapine-Specific T Cells from Drug-Naive Individuals and Patients with Agranulocytosis

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Clozapine, an atypical antipsychotic previously withdrawn due to agranulocytosis, was re-introduced due to the lack of efficacious alternatives. Extensive monitoring of patients' neutrophil counts before, during and after therapy, suggests an unresolved drug safety issue to which the molecular mechanisms are poorly understood. The delayed onset agranulocytosis (3-4 weeks) after the initial clozapine exposure, rapid onset of symptoms upon rechallenge and the association of HLA-DQB1 and HLA-B variants suggest an immunological mechanism. The aims of this study were (i) to generate clozapine/metabolite-specific T-cell clones from HLA typed clozapine-naïve individuals and patients with agranulocytosis by serial dilution and mitogen stimulated expansion and (ii) to characterise the molecular mechanisms and pathway(s) involved in T-cell activation, using functional assays, including cytokine secretion and proliferation, and for cross reactivity studies with *N*-desmethylclozapine, clozapine *N*-oxide and olanzapine. Five clozapine-specific CD4+ clones and one CD8+ clone were generated from patients and clozapine naïve individuals; activation required the presence of antigen presenting cells, with clozapine interacting directly with MHC class II or MHC class I. Clones secreted a combination of Th1 (IFN-gamma), Th2 (IL-5, IL-13), Th22 (IL-22) and IL-10 (anti-inflammatory) cytokines, as well as cytotoxic molecules (Granzyme B and Fas ligand), and expressed 3 distinct T-cell receptors (TCR Vβ 5.1, 20 and 22) and CXCR3, CCR6, CCR4, CCR9, CCR8 and E-cadherin. Cross reactivity with N-desmethyl clozapine and olanzapine, but not with clozapine *N*-oxide was observed. Clozapine interacted with several HLA-DR molecules, including HLA-DRB1*07:01 and HLA-DRB1*15:01 to activate the CD4+ clones. *In silico* molecular docking with HLA-DRB1*15:01 revealed that clozapine, N-Desmethyl clozapine and olanzapine appear to bind in a similar confirmation to the P4 and P6 peptide binding pockets. Clozapine *N*-oxide occupied the P6 pocket in a different binding orientation. This study is the first to characterize clozapine-specific T-cells from patients and drug-naïve individuals. Additional work is required to delineate their precise role in the pathogenesis of clozapine-induced agranulocytosis.

PS 1875 Characterization of T Cell Responses in Patients with Liver Injury following Treatment with the BACE Inhibitor Atabecestat

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Atabecestat is an orally administered BACE inhibitor which was in development for the treatment of Alzheimer's disease. Patients in clinical studies with atabecestat exhibited elevations in hepatic ALT and AST, without a suggestion for cholestasis. In most cases ALT/AST levels reduced upon cessation of atabecestat treatment, however in a limited number of patients ALT/AST levels continued to increase following cessation of atabecestat treatment prior to an eventual reduction. The delayed onset of liver enzyme elevation suggests an immune pathogenesis; thus, the aim of this study was to generate and characterize atabecestat and atabecestat metabolite-specific T-cells from patients with liver injury. PBMC were isolated from the whole blood of fourteen patients selected from atabecestat clinical studies. From the 14 selected patients, 12 exhibited elevations in liver enzymes. These cells were first subjected to the lymphocyte proliferation and ELIspot assays in the presence of atabecestat and metabolites (diaminothiazine [DIAT], *N*-acetyl-DIAT and epoxide). Furthermore, T-cell lines generated against atabecestat and metabolites were subjected to serial dilution to generate T-cell clones. Phenotypic analyses were conducted using flow cytometry, while functional studies characterized cross-reactivity, cytokine release and pathways of T-cell activation. Despite negative PBMC proliferation and ELIspot results, seventeen drug-responsive, primarily CD4+, T-cell clones were generated from five atabecestat DILI patients. Clones displayed reactivity against atabecestat (n=4), DIAT (n=10) and *N*-acetyl-DIAT (n=3). The drug-responsive CD4+ clones were activated in an HLA class II-restricted manner and displayed a Th1/2 cytokine secretion profile. All clones were activated through a direct interaction with HLA, with no requirement for antigen processing. Finally, a subset of atabecestat- and DIAT-responsive clones cross-reacted with the *N*-acetyl-DIAT metabolite. In conclusion, the detection of atabecestat(metabolite)-responsive T-cell clones from patients with DILI is indicative of an immune-mechanism for the observed hepatic enzyme elevations.

PS 1876 The Incidence of Infusion Reactions Associated with Monoclonal Antibody Drugs Targeting the Epidermal Growth Factor Receptor in Metastatic Colorectal Cancer Patients: A Systematic Literature Review and Meta-Analysis of Patient and Study Characteristics

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Infusion reactions have been reported in studies of metastatic colorectal cancer (mCRC) patients treated with anti-EGFR therapies, including cetuximab and panitumumab, with incidences ranging from 0-33%. A systematic literature review and meta-analysis were conducted to estimate the incidence of infusion reactions in this population and identify variations in this incidence by patient and study characteristics. Multiple scientific databases were searched to identify observational studies or clinical trials of mCRC patients treated with anti-EGFR therapies that reported rates of infusion reactions, hypersensitivity, or allergy/anaphylaxis. Random effects models were used to meta-analyze the incidence of infusion reactions overall and stratified by therapy, study design, geographic location, *KRAS* mutation status, and grade of reaction severity. Among 48 studies included in this meta-analysis, the pooled estimate for infusion reaction incidence was 0.049 (95% CI: 0.036 - 0.065), or nearly 5%. Reactions of grades 1 or 2 were more common than reactions of grades 3-5 (0.089 vs. 0.028) No significant variations in infusion reaction incidence were observed by study design, KRAS status, or study location. Infusion reactions occur in approximately 5% of mCRC patients treated with anti-EGFR therapies and the incidence varies significantly by grade and severity. Future studies should consider investigating survival outcomes for only those patients with infusion reactions to determine its prognostic relevance.

PS 1877 Delineation of the Role of Gut Microbiome in Trichloroethene-Mediated Autoimmunity

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Exposure to trichloroethene (TCE) is associated with autoimmune diseases (ADs), including systemic lupus erythematosus (SLE) and autoimmune hepatitis (AIH). However, mechanisms contributing to TCE-mediated autoimmunity are not well-established. Although gut microbiome dysbiosis has been implicated in the pathogenesis of ADs, whether TCE induces gut microbiome modification and if it is a causal factor or an outcome of the disease remains unknown. This study was, therefore, focused on determining (1) the composition of gut microbiome in female MRL+/+ mice at 24 and 36 weeks (W) post TCE exposure, and (2) the involvement of gut microbiome in lupus pathogenesis using unique SLE mouse models with varying degrees of disease progression (C57BL/6 as negative control, SLE-prone MRL+/+ with slow disease development or MRL-lpr mice with aggressive disease). Feces collected from these mice at 6 and 18 W as well as TCE-treated mice were subjected to 16S rRNA sequencing to determine the microbiome composition. Intestinal barrier functions and mucosal immune changes were analyzed by testing colonic tight junction proteins, cytokines, IgG production, fecal albumin and IgA levels. Serum autoantibodies and histology were evaluated to monitor disease progression. Our data showed that TCE exposure had distinct bacterial community revealed by β-diversity, and was accompanied by increases in phylum *Verrucomicrobia* at 24 and 36 W. TCE exposure also led to increased antinuclear antibodies (ANA) at 36 W. Studies using three mouse strains showed a lower Firmicutes/Bacteroidetes ratio in 6 W MRL-lpr mice, evidenced by a reduction in abundance of *Clostridiales* and significant increase in *Rikenellaceae* families. In 18 W MRL-lpr mice, altered microbiome composition was associated with increased gut permeability, inflammation, and AD markers (ANA and ASMA), along with immune cell infiltration in liver and severe glomerulonephritis. Our data indicate that TCE exposure leads to

gut dysbiosis and autoimmunity in MRL+/+ mice, and mechanistic studies showed that microbiome dysbiosis was associated with increased gut permeability, inflammatory responses and autoimmunity markers of SLE/AIH-like diseases. Our findings clearly indicate a role of gut dysbiosis in ADs and provide an intriguing mechanism for TCE-induced autoimmunity.

1878 Evaluation of Contact Sensitization Induced by Four Ionic Liquids

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Ionic liquids (ILs) are synthetic solvents used in chemical production processes and have been labelled as "green" replacements for volatile organic solvents. Recent reports indicate that ILs may induce oxidative stress, dermal toxicity, irritation, and sensitization in rodents which is a concern because human exposure is anticipated to occur via dermal or oral routes. The objective of this study was to assess the contact sensitization potential of four common ILs: 1-ethyl-3-methylimidazolium chloride (EMIM, dose 6.25-50% v/v), 1-butyl-3-methylimidazolium chloride (BMIM, dose 3.12-12.5% v/v), 1-butyl-1-methylpyrrolidinium chloride (BMPY, dose 0.825-6.25% v/v), and N-butylpyridinium chloride (NBuPY, dose 0.825-12.5% v/v). The test article, or vehicle control, was applied to the dorsa of both ears of female Balb/c mice daily for three days in a combined local lymph node (LLNA)/primary irritancy assay. In the mouse ear swelling test (MEST), the mice were sensitized on the back for 3 days, rested for 4 days, and challenged on the right ear. Sensitization was also assessed *in vitro* in the Direct Peptide Reactivity Assay (DPRA), the KeratinoSens™ Assay, and the Human Cell Line Activation Test (hCLAT). EMIM was neither an irritant nor a sensitizer in any assay. *In vivo*, BMIM and BMPY were not irritants. However, dermal treatment with 12.5% BMIM or 6.25% BMPY induced an increase ($p<0.01$) in lymph node cell proliferation in the LLNA, but the increase did not achieve the SI3 level associated with sensitizers. Sensitization (3.12%) and challenge (6.25%) with BMIM induced an increase ($p<0.05$) in ear swelling (82-183%) in the MEST, however there was no effect following exposure to BMPY. *In vitro*, BMIM was classified as a sensitizer by the hCLAT but was negative in the DPRA and KeratinoSens™ assays. BMPY was classified as a sensitizer by the hCLAT and KeratinoSens™ assay, but negative in the DPRA. NBuPY induced contact irritation *in vivo* at >3.12% ($p<0.01$), and sensitization *in vitro* in the KeratinoSens™ assay and hCLAT, but failed to induce sensitization *in vivo* and was negative for peptide reactivity in the DPRA. In summary, the butylated ILs, BMIM, BMPY, and NBuPY, were more potent than the ethylated EMIM. An integrated analysis of the *in vivo* and *in vitro* data suggests that BMIM and BMPY may induce sensitization at higher concentrations.

1879 Identify Structure Alerts Associated with Drug-Induced Autoimmune Diseases

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Drug induced autoimmune diseases is an adverse event that could lead to severe clinical outcome. Due to its complicated pathogenesis, no effective approach is available to assess a drugs' liability to induce autoimmune disease. Some drugs with specific substructures were frequently observed to cause autoimmune diseases. Here, a data mining approach was developed to identify structure alerts associated with the possibility to cause autoimmune diseases. 61 drugs were assigned as the culprit with evidence causing autoimmunity and another set of 593 drugs was defined as negative after filtering out 46 autoimmunity preferred terms in their drug labels. Furthermore, a maximum common substructure (MCS) method was applied in this dataset and 10 structure motifs that were statistically significantly associated with presence of autoimmune diseases were identified. Some motifs (e.g. quinoline) were reported associated with the high possibility to generate reactive metabolites, a mechanism that induces hypersensitivity and immune responses. The structure alerts identified here could be applied to screen out future drug candidates with the potential to cause autoimmune diseases. Further validation is warranted.

1880 The Androgenic Impact of AKR1C3 and 11-Ketoandrogens on Polycystic Ovarian Syndrome (PCOS)

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Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy in women of reproductive age affecting 10% of all women and at least 30% of all infertile women. PCOS is characterized by hyperandrogenism and leads to obesity and type 2 diabetes. Our goal is to identify targets for endocrine disrupting chemicals (EDCs) that could influence this disease. Aldo-keto reductase family 1 member C3 (AKR1C3; type 5 17β-hydroxysteroid dehydrogenase (HSD) and prostaglandin F synthase) is the prominent peripheral enzyme involved in androgen biosynthesis and the production of prostaglandins of the F series. AKR1C3 overexpression in adipocytes in response to insulin could lead to increased androgen receptor (AR) signaling, increased *de novo* lipogenesis, and decreased PPARγ signaling leading to reduce adipogenesis. This lipid overflow could exacerbate insulin resistance through a feedforward mechanism and promote the lipotoxic profile seen in PCOS. In adipocytes, the 11-oxygenated androgens of adrenal origin may be a potential source of peripheral androgens leading to the androgen excess. Serum samples from PCOS patients have increased levels of 11β-hydroxyandrostenedione (11βOH-A4), 11-ketoandrostene-3,17-dione (11K-A4), and 11-ketotestosterone (11K-T). 11K-T and 11-ketodihydrotestosterone (11K-5α-DHT) have the same potency as testosterone and dihydrotestosterone in AR reporter gene assays. To determine whether AKR1C3 can convert 11K-A4 to 11K-T and 11-keto-5α-androstane-3,17-dione (11K-5α-dione) to 11K-5α-DHT, kinetic parameters for these reactions catalyzed by recombinant AKR1C3 were estimated using discontinuous UV RP-HPLC enzymatic assays. Detection of UV transparent compounds was accomplished by dinitrophenylhydrazine derivatization. AKR1C3 was found to have catalytic efficiencies for 11K-A4 and 11K-5α-dione that were 5 times greater than its normal substrate A4. By contrast 11βOH-A4 was found to be a poor substrate indicating that it must be converted to 11K-A4 first by 11β-HSD2. The kinetic analysis helps to elucidate the involvement of AKR1C3 and 11β-HSD2 in the formation of the 11-oxygenated androgens. The study identifies AKR1C3, 11β-HSD2, AR and PPARγ as targets for EDCs that may affect PCOS onset.

1881 Exploring Reproductive Toxicological Dosing Schemes: Effects of Environmentally Relevant Dibutyl-Phthalate Exposures on Terminal Estrous-Stage Gene Expression

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Dibutyl phthalate (DBP) is a plasticizer commonly used in cosmetics and oral medications which have been detected in human urine and ovarian follicular fluid. In women, phthalates are associated with follicle loss, infertility and early menopause. Previous studies analyzing phthalate-induced ovarian toxicity have exposed mice for a specific number of days resulting in collection of ovaries at variable terminal stages of the mouse estrous cycle (e.g. estrus vs. diestrus), yet this does not account for sensitivity of genes to estrous changes during chemical exposures. Previously, following an estrous dosing regimen we have shown that DBP does not alter the folliculogenesis and steroidogenesis related genes, insulin-like growth factor 1 (*Igf1)* and aromatase (*Cyp19a1),* in estrus ovaries. We hypothesized that ovarian gene expression will be sensitive to *in vivo* DBP treatment according to terminal stage at collection. To test for differences between stages and treatments we dosed CD-1 females for 20-25 days (equivalent to ~4 estrous cycles) with environmentally relevant doses of DBP (10 and 100 µg/kg/d) and a classical high dose (1000 mg/kg/d) to measure ovarian *Cyp19a1* and *Igf1*. Treatment alone had no effect on *Igf1* and *Cyp19a1* mRNA, but higher DBP treatments of 100 µg/kg/d and 1000 mg/kg/d caused differential *Igf1* mRNA expression between metestrus/diestrus stages compared to proestrus/estrus. Interestingly, uterus weight was significantly decreased in the proestrus/estrus ovaries only with all DBP treatments, a difference that was not observed at metestrus/diestrus. There were no differences in other tissue weights or overall weight gain in response to DBP exposure. These results reveal that *Igf1* and uterus weight sensitivity to DBP exposure varies according to terminal stage. These findings support the idea that analysis throughout the estrous cycle should be considered when using female mouse models in reproductive toxicological studies. *Supported by NIH R01ES026998.*

1882 Aldosterone Secretion in a Human Adrenal Cell Line Is Enhanced by the Endocrine-Disrupting Chemical Di-(2-ethylhexyl) Phthalate (DEHP)

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Di-(2-ethylhexyl) phthalate (DEHP) is an endocrine-disrupting chemical that is widely used as a plasticizer in the production of polyvinyl chloride containing plastics. DEHP is a component of many common household items (toys, packaging, furniture, etc.) and medical devices, resulting in significant and chronic human exposure. Human exposure to DEHP has been linked to androgen disruption and developmental toxicity, including cryptorchidism, decreased anogenital distance, and decreased testosterone levels. Other adrenal corticosteroids are also susceptible to the endocrine-disrupting effects of DEHP. One study has demonstrated that fetal exposure to DEHP results in altered adrenal aldosterone production in adult rats. Due to these potential effects of DEHP on adrenal cortex steroidogenesis, the focus of our studies is to characterize the effect of DEHP on aldosterone secretion in a human adrenocortical cell line. HAC15 cells were exposed to vehicle or various concentrations of DEHP (10 nM-30 μM). After 72 h, cell viability, aldosterone secretion, and gene expression of enzymes and cofactors involved in aldosterone synthesis was examined. No effects on cell viability were observed with the concentrations studied. Basal aldosterone secretion was significantly increased from 100 nM-30 μM DEHP, with maximal secretion at 10 μM (in pg/mL: vehicle, 75.6±3.5; 10 μM DEHP, 7735.2±151.3; n=8). Ang II-stimulated aldosterone secretion was significantly increased from 100 nM-30 μM DEHP, with maximal secretion at 10 μM (in pg/mL: vehicle, 4278.9±110.2; 10 μM DEHP, 18651.7±572.2; n=8). ACTH-stimulated aldosterone secretion was significantly increased from 100 nM-30 μM DEHP, with maximal secretion at 10 μM (in pg/mL: vehicle, 124.0±3.7; 10 μM DEHP, 23455.8±272.7; n=8). Gene expression of most enzymes and cofactors involved in aldosterone synthesis were increased by 10 μM DEHP. Aldosterone synthase (CYP11B2) had the greatest induction at 175.2±2.0 fold. These data support the evidence that DEHP disrupts the regulation of aldosterone secretion and provides further evidence that DEHP is an endocrine disruptor.

1883 *In Vitro* Impact of the Endocrine Disruptors Genistein and Mono–(2–Ethylhexyl) Phthalate (MEHP) on the Eicosanoid Pathway in Spermatogonial Stem Cells

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Perinatal exposure to endocrine disrupting compounds (EDCs) at environmentally relevant doses can alter the male reproductive system. Infants may be exposed to the EDC genistein (GEN), an isoflavone found in soy-based infant formulas, and MEHP, metabolite of the plasticizer DEHP, prevalent in plastics from medical equipment. We have shown that fetal exposure of rats to GEN and DEHP mixtures altered germ cell markers and increased infertility and abnormal testis phenotypes. In addition, mast cell and macrophage markers were also altered, which is indicative of inflammatory events in testes and can be linked to infertility in men. Inflammation is mediated by the eicosanoid biosynthesis pathway, where prostaglandins (PGs) are synthesized via several enzymatic steps starting with the release of arachidonic acid from membrane phospholipids. The rate limiting enzymes, cyclooxygenases (COXs), are associated with pain, fever and chronic inflammation. Moreover, PGs regulate physiological processes such as cell growth, female reproduction and fertilization. We propose that GEN and MEHP disruption of the eicosanoid pathway in germ cells might contribute to their adverse effects. The mouse C18–4 cell line is a model for undifferentiated spermatogonia, including spermatogonial stem cells (SSCs) which expresses similar eicosanoid pathway genes as found in primary rat spermatogonia by gene array. The cells were treated with GEN and/or MEHP at 10^{-5} or 10^{-4} M for 24 hours, with normal or charcoal-stripped 10% fetal bovine serum. While *Cox2* gene expression was increased by 10^{-4} M GEN and GEN+MEHP, *Cox1* mRNA was decreased with 10^{-5} M and 10^{-4} M GEN and GEN+MEHP treatments. Moreover, EDC-treated cells produced higher levels of PGD2 than controls. The transcripts of the undifferentiated spermatogonia markers *Foxo1* and *Mcam* increased, indicating remodeling of spermatogonia transcriptome by GEN and the EDC mixture. Silencing COX1 expression with shRNA resulted in cells expressing higher levels of the differentiated spermatogonia marker *cKIT*, suggesting a role of COX1 in maintaining spermatogonia in an undifferentiated state. Thus, GEN and MEHP exposure might disrupt spermatogonia status by altering COX1 expression. These data suggest that exposure to environmentally relevant doses of GEN and GEN+MEHP can disrupt the eicosanoid pathway and interfere with the mechanisms regulating self–renewal and differentiation processes in SSCs.

1884 Exposure to Acetaminophen Is Associated with Modified Aromatase Activity and Estrogen Synthesis in Human Placenta JEG-3 Cells

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Acetaminophen is the most widely used drug for the treatment of pain and fever. It is taken by nearly two-thirds of pregnant women, making it the most commonly used over-the-counter medication during pregnancy. Of concern, several recent studies have reported an association between *in utero* acetaminophen exposure and adverse birth outcomes including cryptorchidism, short anogenital distance and reproductive abnormalities. Additionally, acetaminophen has endocrine disrupting properties and has been shown to reduce testosterone production in human fetal testes. As the placenta is an endocrine organ and a mediator of maternal-fetal interaction, exposure to acetaminophen during pregnancy could perturb placenta morphology, physiology, gene expression and epigenetic machinery. Highlighting a fundamental research gap, the effects of acetaminophen on the placenta have never been thoroughly studied. The goal of this study was to determine the effect of acetaminophen on placental steroidogenesis. Human placental JEG-3 cells were exposed to clinically-relevant concentrations of acetaminophen for 24 hours. mRNA expression as well as protein levels of steroidogenic genes were assessed. Acetaminophen was associated with decreased mRNA and protein levels of aromatase (CYP19A1), an enzyme involved in the conversion of androgen to estrogen. The results of the present study highlight that acetaminophen may potentially modulate estrogen levels by inhibiting CYP19A1 activity in the placenta.

1885 The Enhanced Predictive Assessment of Effects on Steroidogenesis (OECD TG 456) by Analyzing a Broadened Steroid Panel by LC-MS/MS

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The regulatory accepted method to identify compounds that interfere with the steroid hormone system claims quantitative determination of testosterone (T) and estradiol (E2) in supernatant of human adreno-carcinoma cell line H295R (OECD TG 456). H295R is the cell line of choice, as it harbors the genes encoding key enzymes for steroidogenesis. Changes in estradiol and testosterone levels in the supernatant of treated cells are detected by the assay indicating a potential unwanted impact of the dosed substance on E2 and T. The aim of toxicology scientist worldwide is the determination of endocrine disruptions with the highest reliability in an *in vitro* approach. For that reason, the analytics for E2 and T was further developed from the classical immunoassays approach to analytical methods such as LC-MS/MS, minimizing the risk of cross-reactivity of the test substance with the test system. The next evident development step on the way to a best possible recognition rate of effects on the endocrine system is the broadening of analytes covered by the analytical system, which becomes important in the corresponding hormone pathways. Many additional improvement steps have been taken, both in analytics (e.g. online solid phase extraction, chemical derivatization) and cell culturing (e.g. enhanced growth conditions), to optimize this important assay. Here we show the results of our longstanding technical development of the enhanced steroidogenesis assay, based on the initial approach described in the OECD TG 456. Additionally, we share our experience out of more than 10.000 measurements of individual H295R-supernatant samples. The enhanced online LC-MS/MS method delivering absolute quantitative results from 16 hormones relevant in this context is described in detail to share with the scientific community. Analytical quality is shown androstenedione, testosterone, progesterone, deoxycorticosterone, deoxy cortisol, cortisol, estradiol, estrone, cortisone, hydroxyprogesterone, dehydroepiandrosterone, hydroxy pregnenolone, estriol, pregnenolone, 18-hydroxy deoxycorticosterone and corticosterone by validated values for the sensitivity and the reportable range (lower/upper limits of quantitation, LLOQ, ULOQ) for each individual analyte completed by accuracy and precision. Examples for the enhanced potential in identification and prediction of endocrine disruptive events will be discussed.

1886 Bisphenol A Induced Steroidogenic Enzyme Hsd17b2 through Activation of Orphan Nuclear Receptor Err Gamma V1

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Endocrine disruptors interfere with the normal functioning of the endocrine system. Bisphenol A (BPA) is a widely used industrial plasticizer with known endocrine disrupting property. Environmental exposure to BPA and their accumulation in human body present toxicological concerns. Over the past several decades, male reproductive functions have been declining in human and wildlife. The present study aims to investigate whether exposure to BPA adversely impacts the functions of the male reproductive system. Methods: Two commonly used mouse Leydig cell lines (I-10 and MLTC-1) were screened to determine whether BPA exposure would alter the expression of key enzymes involved in testicular steroidogenesis. Then, a correlation between the expression of orphan nuclear receptors Err family members and the induction of steroidogenic enzymes in response to BPA were assessed. Furthermore, overexpression of Err family members was employed to assess their direct impact on BPA-responsive steroidogenic enzymes. BPA concentration-dependently induced Hsd3b6 expression in both I-10 and MLTC-1 cell lines. I-10 cells seemed to be more sensitive to BPA exposure than MLTC-1 cells, as two additional steroidogenic enzymes, Cyp17a1 and Hsd17b2, were also concentration-dependently induced by BPA only in I-10 cells. Correlating to the differential BPA responsiveness between the two cell lines, two Err family members, alpha and gamma V1, have higher basal expression levels (2.9-fold and 8.7-fold, respectively) in I-10 cells compared to MLTC-1 cells. In addition, Err gamma V1 was markedly induced by BPA in I-10 cells in a concentration-dependent manner. Furthermore, overexpression of Err gamma V1, but not other Err family members, caused further induction of Hsd17b2 in response to BPA treatment. BPA induced the expression of Hsd3b6, Cyp17a1, and Hsd17b2 in Leydig cells, all of which are conserved and key enzymes in steroidogenesis. In addition, the induction of Hsd17b2 by BPA seemed to depend on the orphan nuclear receptor Err gamma V1. Taken together, our findings suggest that BPA may serve as a novel ligand for the orphan nuclear receptor Err gamma V1 and through its activation BPA up-regulates Hsd17b2 expression.

1887 Chemicals That Increase Synthesis of Estradiol and Progesterone Are Potential Risk Factors for Breast Cancer

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Established breast cancer risk factors such as hormone replacement therapy and reproductive history are thought to be related to increased estrogen and progesterone levels or activity. While most research about endocrine-active chemicals has focused on chemicals that bind to and activate the estrogen receptor, little attention has been paid to chemicals that may affect steroidogenesis, for example by increasing synthesis of estradiol or progesterone systemically or in the breast. Using publicly available data from EPA's ToxCast, we identified chemicals that increased levels of estradiol (E2-up) and/or progesterone (P-up) in a H295R steroidogenesis assay -which uses adrenal cortical cells to measure chemical effects on steroidogenesis *in vitro*. We prioritized active chemicals based on potency using their lowest active concentration (LAC), ToxCast AC50s, and an integrated measure of effects on the steroid synthesis pathway (adjusted maximum mean Mahalanobis distance, or adj.maxmMd). We further prioritized chemicals based on expected general population exposures using EPA's high throughput exposure modeling. Based on the ANOVA at each dose, OECD hit call logic, and limiting to chemicals with adj.maxmMd greater than zero, 270 chemicals increased E2 and 280 increased P, with 139 increasing both. Of these chemicals, 161 (54 E2-up, 125 P-up) were also identified as positive using ToxCast's data processing pipeline. The rest included 22 E2-up and 35 P-up chemicals with LAC less than 1 micromolar and 136 E2-up and 84 P-up chemicals with LAC between 1 and 33 micromolar. Median general population exposure predictions are above 0.01 mg/kg-day for 59 of the 270 E2-up and 47 of the 280 P-up chemicals. *In vivo* outcomes reported for some of these chemicals include mammary gland tumors and effects on vaginal opening as might be expected, but *in vivo* confirmation is hindered by lack of relevant endpoints in guideline studies and incomplete reporting of mammary gland effects. ToxCast data processing appears to obscure some non-monotonic responses in this assay. This work highlights chemicals that are a priority for exposure reduction, biomonitoring, and further study of health effects based on their ability to increase levels of estradiol and progesterone. Effects on breast development and breast cancer risk are of special interest. Current toxicity guideline studies do not include adequate endpoints that are sensitive to effects on mammary gland, and so additional assessments and more comprehensive reporting are needed to detect these effects.

1888 Bisphenol A and Bisphenol F Exposure Alters Brood Size and Decreases Food Availability for *C. elegans*

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Bisphenol A (BPA) is a commonly used chemical in the synthesis of polycarbonate plastics. Because of growing concern for the estrogenic and neurological effects, some federal governments have implemented strict regulations on the use of BPA, which have increased the use of other common bisphenol analogues. Due to the structural similarity, Bisphenol F (BPF) is hypothesized to induce reproductive toxicity similar to BPA. Previous bisphenol toxicity studies have been conducted in polystyrene petri dishes. While companies (VWR, ThermoFisher) report no BPA in their petri dishes, bisphenol compounds may be leaching into the media. To eliminate this potential confounding factor, we utilized glass petri as an additional control. Wild type *Caenorhabditis elegans* were exposed to 250µM or 500µM BPA or BPF for 2 days (concentrations required to provide internal concentrations similar to those observed in human studies). After 2 days, adult hermaphrodites were moved to individual plates for quantification of brood size. All groups displayed a significant decrease in brood size compared to the glass petri dish control group. Specifically, the control plastic group laid 13% less eggs (p= 0.0028) and the BPA and BPF 500µM groups laid 22.1% and 23.9% less eggs (p0.0001); suggesting that BPA and BPF display estrogenic activity and reduced fecundity. *C. elegans* populations are typically 99.99-99.999% hermaphroditic. Male *C. elegans* were observed during treatment, which motivated the development of an experiment to assess the effects of bisphenol compounds on male fertility. The effect of BPA and BPF on the growth of *E. coli*, the food source for *C. elegans,* also indicated that bisphenol exposure altered normal growth. After 24-hour bisphenol exposure, control *E. coli* populations grown in plastic labware cultured a population size 23.61% less than that cultured in glass labware (p0.0001); BPA and BPF 500µM exposure caused a 40.10% and 50.7% decrease in population size (p0.0001). Results from this study indicate that BPF induces toxic effects similar to BPA in *C. elegans* as well as diminishing the viability of *E. coli*. This effect was also seen in the polystyrene petri dish controls, which brings the suitability of their use for bisphenol research. Further research is needed to determine the extent and potential mechanisms of toxicity.

1889 *In Vitro* Bioassay and Screening Model for Pre- and Post-Distribution Chicago-Area Treated Waters

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Recent contamination of urban water supplies have highlighted the importance of screening tap water after drinking water treatment and distribution. Our goals were to efficiently screen for potential sources of estrogenic, androgenic and glucocorticoid receptor-active compounds in water samples from residential sites in the greater Chicago, Illinois metropolitan area. Sampling sites were based on community volunteers and were selected for broad coverage of the Chicago Department of Water Management service areas. Known endocrine active compounds were measured in water samples using LC/MS-MS and endocrine activity was measured using luciferase reporter transcriptional activation (TA) and chemiluminescent yeast (CLY) bioassays. Tap water samples were not expected to produce bioassay responses great enough to accurately estimate biological equivalency concentrations. Therefore, we screened sample extracts initially to identify the samples with detectable concentrations of activity and then performed a secondary dose-response screen to generate sample extract EC_{50} values. Estrogenic activity was detected above method detection limits, in untreated water samples only, using the TA (5/39 sites; median: 0.21 ng E2Eqs/L) and CLY (5/39; 1.78 ng E2Eqs/L) bioassays. Androgenic activity was detected using a novel TA bioassay in untreated and treated pre-distribution samples (4/39; 0.93 ng DHTEqs/L), but there were no androgenic activity detects using the CLY. No glucocorticoid activity was detected above method detection limits in either bioassay. Known estrogen receptor agonists estrone (0.72-1.4 ng/L), 17α-estradiol (1.3-1.5 ng/L), and 17β-estradiol (1.4 ng/L) were detected in a limited number of extract samples and did not necessarily correlate with estrogen TA and CLY bioassay detections. Overall, we applied a new and highly sensitive method for detecting androgenic activity and provide a tiered model for tap water bioassay screening that increases screening speed without increasing the false negative rate. These novel and highly sensitive approaches are necessary especially while screening low activity generally present in tap water samples. *Abstract does not necessarily reflect US EPA policy.*

1890 Computational Identification and Analysis of Nonmonotonic Concentration Responses in Tox21 Estrogen Receptor Assays

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Environmental endocrine disrupting chemicals (EDCs) interfere with the metabolism and actions of endogenous hormones. It has been well documented that EDCs can exhibit nonmonotonic dose response (NMDR) behaviors in numerous *in vivo* and *in vitro* studies. Not conforming to the conventional linear or linear no threshold response paradigm, these NMDR relationships pose practical challenges to the risk assessment of EDCs. Yet, the endocrine signaling pathways and biological mechanisms underpinning NMDR remain incompletely understood. The US Tox21 program has developed *in vitro* cell-based assays for estrogen receptors (ER), androgen receptors (AR), and other nuclear receptors, and screened 10K chemicals for potential agonist or antagonist activities in a high-throughput screening platform. Containing fifteen concentration points across several orders of magnitude of concentration range and run in both agonist and antagonist modes, these Tox21 assay data contain valuable quantitative information that can be explored to evaluate the nonlinearities in the effects of EDCs and potential cellular mechanisms. In this study we analyzed the concentration-response curves from the Tox21 screening data of ERα and ERβ assays in both agonist and antagonist modes as well as cell viability readout. We used both unsupervised and supervised machine learning algorithms to identify and cluster various shapes of concentration-response curves. The machine-learning method customized to the dataset is able to identify hundreds of NMDRs with high accuracy and high confidence. Even after eliminating nonmonotonic responses likely caused by cytotoxicity or auto-fluorescence, significant numbers of U- and inverted U-shaped concentration-response curves remain in both the agonist and antagonist assays. For a given chemical, the unique concentration-response profile appearing in both agonist and antagonist assays provides important insights into the potential pathway mechanisms that may underpin the observed nonmonotonic response. These profiles can be better understood via dynamic modeling of the interplay between the ligand, receptor monomer and dimer, DNA promoter, coactivator, and corepressor. The developed computational methods for NMDR identification in ER assays can be applied to other high-throughput biological response assays.

1891 Chronic Exposure to Bisphenol S Causes Thyroid Hormone Disruption and Reproductive Dysfunction in Zebrafish with Sex Difference

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Zebrafish (*Danio rerio*) has been used as a model for behavioral research, genetics, physiology, disease modeling, drug discovery, and toxicology due to similarity of tissue-specific physiological processes between zebrafish and humans. Metabolic study using zebrafish provide important information regarding molecular-level effects and toxicity of xenobiotic chemicals. Bisphenol S (BPS) is commonly used in curing fast-drying epoxy resin adhesives. The aim of the present study was to examine whether chromic exposure to BPS disrupts thyroid endocrine system and causes reproductive dysfunction in zebrafish. In this study, zebrafish was chronic exposed to different concentrations of BPS (8, 40, and 200 μg/mL) for 21 days. To investigate the metabolic alteration induced by BPS, thyroid hormones and steroids in the whole-body of zebrafish analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after sample preparation steps. In both female and male zebrafish treated with BPS, whole-body T3 levels were significantly increased. In female zebrafish treated with BPS, estradiol (BPS 8 μg/mL; $P < 0.005$, 40 μg/mL; $P < 0.005$, 200 μg/mL; $P < 0.003$) and progesterone (BPS 40 μg/mL; $P < 0.01$, 200 μg/mL; $P < 0.03$) were significantly increased. In male zebrafish treated with BPS, estradiol (BPS 8 μg/mL; $P < 0.002$, 40 μg/mL; $P < 0.004$, 200 μg/mL; $P < 0.01$) was significantly increased as in female zebrafish treated with BPS. On the other hand, testosterone (BPS 8 μg/mL; $P < 0.03$, 40 μg/mL; $P < 0.02$, 200 μg/mL; $P < 0.01$) and 11keto-testosterone (BPS 40 μg/mL; $P < 0.05$, 200 μg/mL; $P < 0.03$) were significantly decreased in male zebrafish treated with BPS. These results demonstrate that chronic exposure to BPS alters whole-body contents of thyroid hormones and steroidogenesis related to reproduction with sex difference, thus exerting reproductive toxicity.

1892 Validation of a Multiplex Assay to Detect Thyroid Hormones in Rat Serum

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Endocrine disruptors are substances that can cause harmful effects to the endocrine system. Thyroid hormones, triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) are involved in development of central nervous and skeletal systems. Test guidelines on the screening and testing of chemicals published by the Organisation for Economic Co-operation and Development (OECD) have been updated to include thyroid hormone measurements for assessing endocrine disrupter potency. It's therefore important that non-clinical toxicology studies are able to provide data assessing potential adverse effects on endocrine endpoints. Several guidelines require analysis of circulating levels of thyroid hormones in juvenile rats. Sample volume and assay sensitivity can become limiting factors. Traditional enzyme-linked immunosorbent assays (ELISA) require relatively large sample volumes and suitability can be limited by poor sensitivity, inaccuracy, and biologically irrelevant assay ranges. The Rat Thyroid Hormone Panel (Merck), enables multiplexing of thyroid hormones in a single analysis, minimising required sample volumes. The panel operates using internally colour coded microspheres, coated with a specific capture antibody. Sample analyte is captured by the bead and biotinylated detection antibody is introduced. The reaction mixture is finally incubated with Streptavidin-Phycoerythrin conjugate. Each microsphere is identified, and the result quantified based on fluorescent reporter signals. A validation was performed evaluating kit performance in both multiplex and singleplex protocols for the relative quantification of T3, T4 and TSH in rat serum. Data capture was performed using a Luminex MAGPIX and xPONENT software. Method limitations for standard parameters were investigated. The panel was acceptable with respect to parallelism, intra and inter run precision, accuracy, recovery, carry-over and calibration standards. The functional range determined was: 194.0 to 10,000.0 pg/mL (T3), 751.6 to 100,000.0 pg/mL (T4), 15.8 to 4,000.0 pg/mL (TSH) and were suitable for the analysis of changes in circulating levels of endogenous analyte. The validation facilitated sample volume reduction to 12μl for measurement of 3 analytes simultaneously, reducing sample volume requirements by 90%. The method is fit-for-purpose for measuring relative serum concentrations of T3, T4 and TSH in support of toxicological studies with acceptable precision and accuracy.

1893 Sexually Dimorphic Thyroid Gene Expression Can Lead to Sex-Specific Responses to Thyroid-Disrupting Compounds

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The thyroid hormone (TH) system is central to brain development and is susceptible to perturbation by environmental chemicals. Testing of chemicals for TH disruption is often done in animals, but to allow for the use of alternative test methods, mechanism of effects must be better described. *Ex vivo* thyroid culture is a tractable model, as it can be used both as an intermediate model system and for characterizing mechanisms-of-action. In this project, we aim to characterize sex differences in thyroid development and developmental windows of TH system vulnerability to chemical exposure to facilitate better design and interpretation of *ex vivo* thyroid culture. Initial studies using explanted rat fetal thyroids exposed for 72 and 120 h to 100 μM propylthiouracil (PTU) revealed that i) key genes are dysregulated by PTU exposure and ii) the thyroids have sex-specific transcriptional profiles that give rise to sex-specific responses to chemical insult. Genes, including *Tshr* and *Nkx2-1*, displayed sexually dimorphic expression patterns after 72 h culture, a sex difference that disappeared after 120 h culture. After exposure to PTU, a thyroperoxidase (TPO) inhibitor, we observed compensations in both males and females. In males, these compensatory mechanisms were identified as upregulated *Nis* expression in response to PTU exposure, as well as a trend for upregulated *Tpo* and *Tg* expression with no effects on *Tshr*. In females, there was only a trend towards upregulated *Nis* expression, no effect on *Tpo* and *Tg*, and a downregulation of *Tshr*. These findings suggest sexually dimorphic effects in response to chemical exposure. These sex differences have implications for interpretation of thyroid disruption in animal studies and for further development of test methods for TH disruption.

1894 Use of FRTL-5 Cells in a Secondary Assay following High-Throughput Screening (HTPS) for Sodium Iodide Symporter (NIS) Inhibitors

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Iodide uptake in the thyroid via the sodium-iodide symporter (NIS) is the first step in the biosynthesis of thyroid hormones. Potential NIS inhibiting chemicals were previously screened in our laboratory using a human hNIS-HEK293T-EPA (hNIS) cell line and a radioactive iodine uptake assay (RAIU). Approximately 1800 ToxCast ph1_v2, ph2, and e1K chemicals were screened with this HTPS assay and ranked for potency and cytotoxicity. Because the Fischer rat thyroid follicular (FRTL-5) cell line endogenously expresses NIS, we evaluated an FRTL-5 RAIU as a secondary screening tool to further clarify and prioritize potential NIS inhibitors. Following assay validation, results from controls, reference and chemical tests indicated a highly robust and reproducible secondary assay. Test chemicals included; 30 top-ranked ToxCast chemicals from hNIS HTPS and 10 additional chemicals, which were then tested in parallel FRTL-5, hNIS, and Cell Titer Glo cell viability assays. An orthogonal propidium iodide cytotoxicity assay was also conducted to identify effects on cell membrane integrity. Test chemicals were run at six concentrations (0.001-100µM) in 3 independent biological replicates. The majority of the test chemicals showed IC50 values within one order of magnitude for NIS inhibition. This data demonstrates the utility of a secondary RAIU assay in a more physiologically relevant assay that also provides a species comparison and strengthens the confidence for further prioritization. Several chemicals displaying strong NIS inhibition in both the FRLT-5 and hNIS assays with minimal cytotoxicity will be moved forward for additional testing in short-term *in vivo* assays, including etoxazole, methoxyfenozide, cyprodinil and oxyfluorfen. *This abstract does not necessarily reflect US EPA policy.*

1895 Optimization and Standardization of a Human Microsomes Based DIO Inhibition *In Vitro* Assay

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Thyroid hormones are relevant for metabolic homeostasis and impairment of homeostasis has been associated with several adverse effects. Increased regulatory requirements combined with the need to identify different mode of actions (MoA) will result in increased animal testing. To reduce this need as well as to establish screening models for compound development, relevant *in vitro* assays to identify thyroid active substances are needed. The European Centre for the Validation of Alternative Methods (ECVAM) is evaluating and validating numerous *in vitro* methods focusing on different MoA by cooperating with a network of EU laboratories (EU-NETVAL). One of the important regulators of systemic and local thyroid hormone balance are deiodinases (DIO) by activation of T4 to T3 and degradation of thyroid hormones via deiodination. DIO1, one of the three isoforms, serves as one main source for circulating T3 via deiodination of T4 in liver, kidney and thyroid and has a role in rescuing thyroid hormone bound iodide from biliary excretion. Jointly with the original developer (Charité Berlin), a non-radioactive approach to determine substance-induced DIO1 inhibition based on iodide release in liver microsomes was established. The released iodide was measured via colorimetric change in the Sandell-Kolthoff reaction. Several sources of human microsomes as well as the inhibitory effect of various substances on the DIO activity were investigated for standardization of the test protocol. The testing of several human liver microsomes showed differences in DIO activity which was addressed by microsome-batch specific adjustment of protein quantity resulting in a turnover value of 1,53 pmol iodide/min for further testing. A concentration dependent decrease in DIO activity after incubation with the noncompetitive, specific DIO1 inhibitor 5-Propyl-2-thiouracil (IC50: 2,7 µM; n=5, R^2=0,93) and DIO inhibitor Aurothioglucose (IC50: 0,78 µM; n=5; R^2=0,98) was shown. The DIO inhibitor Xanthohumol which inhibits DIO1 in purified enzyme showed no inhibition of DIO1 activity in human liver microsomes. PTU addition and heat inactivation reduced the iodide release in a similar amount indicating complete DIO inhibition through PTU. In total up to 10 different potential inhibitors were tested to show DIO1 inhibition and assay reproducibility. The optimized and standardized human liver microsome based DIO inhibition assay is a suitable medium-throughput method for testing the DIO-inhibiting properties of chemicals. Further 30 reference items are planned to investigate the predictivity and the suitability for interlaboratory validations as well as regulatory purposes.

1896 Differential Effects of the Agricultural Fungicide Imazalil in Various Experimental Models

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The fungicide imazalil (enilconazole) is designed to disrupt fungal growth by targeting CYP enzymes of the ergosterol biosynthesis pathway. Imazalil may however also affect CYP enzymes in humans, including CYP19 (aromatase) of the reproductive system. In human fetal testis culture (*ex vivo*), imazalil can reduce testosterone production, probably by inhibition of additional enzymes such as CYP17. In line with this, we show that Imazalil markedly inhibits steroidogenesis in the H295R cell line. Based on this, we tested Imazalil for its endocrine disrupting potential in rats. Pregnant Sprague-Dawley rats were exposed to vehicle or two doses of Imazalil (0; 8; 24 mg/kg bw/day) by oral gavage from gestational days (GD) 7 to postnatal day (PND) 16 (n=14-16). An additional cohort was exposed to vehicle or three doses of Imazalil (0; 8; 24; 72 mg/kg bw/day) from GD 7-21, followed by caesarean section (N=4). This cohort was included for kinetic investigations, and to test a higher dose where we previously had seen severe maternal toxicity (dystocia). Despite its known inhibition of key CYP enzymes, we observed no significant changes to androgen sensitive endpoints. Neither anogenital distance (AGD; assessed at GD21 or PND1) nor nipple retention (NR; assessed at PND14) were affected. We found large variations between individual animals with regard to Imazalil concentrations in dams, fetal plasma and amniotic fluid, as well as large variations in intra-testicular testosterone levels. Overall, these individual differences in internal exposure and the lack of anti-androgenic effects *in vivo* indicates more complex toxicokinetic- and toxicodynamic-related issues of Imazalil, leading to differential effects in various experimental models.

1898 Determination of the Antidiabetic Property of the Aqueous Extract of *Biophytum sensitivum* on Streptozotocin-Induced Diabetic Rats

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Diabetes mellitus, a common endocrine disorder of man, is considered one of the major health concerns all over the world. *Biophytum sensitivum* is used traditionally in the treatment of several diseases including diabetes. This study was aimed at investigating the antidiabetic property of *Biophytum sensitivum* aqueous extract on streptozotocin induced diabetic rats. Twenty (20) male albino rats weighing 180g - 200g were divided into five groups of four rats each: Group = A- normal control, B- diabetic control, C- diabetic treated with glibenclamide, D- diabetic treated with 200mg/kg of the extract, E- diabetic treated with 400mg/kg of the extract. Diabetes was induced by intraperitoneal injection of streptozotocin at (55mg/kg) in all groups except the normal control group. The aqueous extract of *B. sensitivum* was administered orally for 14 consecutive days. The blood glucose, albumin, total protein, lipid profile, electrolytes and serum enzymes concentrations were investigated after the administration of aqueous extract of *B. sensitivum*. From the results, diabetic control group showed significant ($p<0.05$) increase in the levels of blood glucose, total cholesterol, triglycerides, LDL, serum liver marker enzymes, serum kidney marker enzymes, direct bilirubin, potassium ions (K^+) while a significant ($p<0.05$) decrease in albumin, total protein, HDL, total bilirubin, indirect bilirubin, sodium ions (Na^+), chloride ions (Cl^-) and bicarbonate ions (HCO_3^-) when compared to the normal control group. Oral administration of aqueous extract of *B. sensitivum* to diabetic rats resulted in a reversal of the above diabetic conditions. However, the 400mg/kg was more effective than the 200mg/kg of the extract. Phytochemical screening of the crude extract of *B. sensitivum* revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Terpenes/steroids, Cardiac glycosides, Balsam, Carbohydrates, Phenols and Resins. In conclusion, *B. sensitivum* aqueous has shown to possess hypoglycaemic, hypolipidemic, renoprotective and hepatoprotective effects, and can therefore be used for the management of diabetes at the said dosage.

1899 Multidrug Resistance-Associated Protein 4 (Mrp4) Is a Novel Genetic Factor in the Pathogenesis of Obesity and Diabetes

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Multidrug resistance protein 4 (Mrp4; encoded by Abcc4) is an efflux transporter known to transport several xenobiotics and endogenous molecules, such as prostaglandins and cyclic nucleotides. We recently identified that the lack of Mrp4 increases adipose tissue and body weights in mice. Interestingly, the expression of Mrp4 in the adipose tissue; and its role in adipose tissue physiology are unknown. The current study was aimed at characterizing these specific roles of Mrp4 using wildtype (WT) and knockout ($Mrp4^{-/-}$) mice. Additionally, we performed adipogenesis experiments using NIH-3T3L1 cells and human pre-adipocytes. Our studies determined that Mrp4 is expressed in the adipose tissue; and that the lack of Mrp4 resulted in adipocyte hypertrophy. In mice, lack of Mrp4 resulted in increased blood glucose and leptin levels, and impaired glucose tolerance. Indirect calorimetry analysis using metabolic cages showed that $Mrp4^{-/-}$ mice have decreased energy expenditure (EE), ambulatory activity (AA) and wheel counts compared to WT mice. Additionally, pharmacological inhibition of Mrp4 function in *in vitro* models resulted in increased adipogenesis and adipogenic gene expression. Lack of Mrp4 in both *in vivo* and *in vitro* increased phospho- cAMP response element-binding protein (p-Creb) protein levels and decreased levels of circulating prostaglandin E metabolites. Increased activation of adipose tissue Creb coupled with decreased plasma prostaglandin E metabolite levels due to loss Mrp4 may have played an important role in the development of obese and diabetic phenotypes in mice. In conclusion, the lack of Mrp4 increases the risk of the development of metabolic diseases such as obesity and diabetes.

1900 Organophosphate Flame Retardant Alterations to Energy Homeostasis in PPARg and ERa KO Mouse Models of Diet-Induced Obesity

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Endocrine disrupting chemicals (EDC) are becoming increasingly prevalent in the environment and many are shown to accumulate within human tissues and interact with endogenous hormone receptors. One such EDC is organophosphate flame-retardants (OPFR). OPFR interact with multiple hormone receptors involved in homeostasis, including estrogen receptors (ER) and peroxisome proliferator-activated receptor (PPAR) γ. We have previously observed that exposure to a mixture of OPFR in adult wild-type (WT) mice induces varying sex-dependent alterations of energy homeostasis, orexigenic and anorexigenic peptide hormones, feeding behavior, and activity. This dysregulation of energy homeostasis can cause an increase in susceptibility to metabolic disorders. In the current study, we repeated the previous adult exposure experiments, this time with knockout mouse strains for ERα (ERKO), and brain-specific knockout of PPARγ (PPARKO). We continued our use of a common mixture of OPFR {tris(1,3-dichloro-2-propyl)phosphate, triphenyl phosphate, and tricresyl phosphate, 1 mg/kg/day each} for 4 weeks continuing the comparison between low-fat diet (LFD, 10% kcal fat) and a high fat (HFD, 45% kcal fat) to generate a diet-induced obesity model. We recorded body weight, crude food intake, body composition, meal patterns, glucose and insulin tolerance, and plasma peptide hormone levels. As in the WT experiments, body-weight gain was increased by HFD for both strains, however, the increased weight-gain for males on a HFD induced by OPFR exposure in WT was absent in both strains, suggesting the lack of these nuclear receptors eliminates OPFR influence on body-weight gain. No weight gain OPFR effects were seen in females. Furthermore, while OPFR altered fat/lean mass in WT male mice, male mice of either KO saw no effects, whereas female ERKO mice exhibited the pattern of male WT OPFR effects. Interestingly, while OPFR decreased feeding efficiency in WT females, in ERKO there was no difference in females, and instead this pattern was now observed in males. Additionally, the OPFR food intake reduction seen in WT was absent in both ERKO and PPARKO. Notably, peptide hormones insulin, leptin, and ghrelin were drastically altered, predominantly in female mice of both KO strains, suggesting both a PPARγ and ERα role in OPFR effects. Lastly, insulin tolerance abolished diet effects in the OPFR exposed ERKO males, while we observed a rescue of HFD effect by OPFR exposure in PPARKO. We are currently processing hypothalamic samples for protein and gene expression. In summary, our data indicates that the influence of adult OPFR exposure on diet-induced obesity is mediated, in part, by ERα and PPARγ.

1901 Metabolic Effects of Endocrine-Disrupting Chemicals: Novel Testing METhods and Adverse Outcome Pathways (EDCMET)

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Sponsor: A. Braeuning, EUROTOX

The term "endocrine disrupting chemical" (ED) mostly refers to compounds that interfere with hormone-related signaling pathways, thereby causing adverse health effects. Recently, the concept of endocrine disruption has been extended to alterations at the metabolic level, which may result for example in obesity, fatty liver disease or diabetes, diseases which represent an increasing worldwide health concern. Currently, no validated methods exist to assess metabolic effects of EDs. Thorough understanding of the molecular mechanisms that lead to adverse metabolic effects of EDs is presently lacking. Starting in early 2019, the European Union has funded the EURION cluster of eight projects on the overarching topic 'New testing and screening methods to identify endocrine disrupting chemicals' within the Horizon 2020 framework, in order to address this unmet need and other gaps in the context of ED testing. One of these projects is EDCMET (Metabolic effects of Endocrine Disrupting Chemicals: novel testing METhods and adverse outcome pathways). The project is coordinated by the University of Eastern Finland, and partners from nine European countries contribute to achieving the project's scientific goals. The main objective of EDCMET is to develop validated *in silico*, *in vitro* and *in vivo* methods to assess metabolic effects of EDs. EDCMET will apply the adverse outcome pathway (AOP) paradigm to identify molecular initiating events (MIE) that can be used for the prediction of emergent adverse biological phenotypes. A strong focus is put on energy and fat metabolism, as well as on nuclear receptors as molecular targets that regulate these processes. EDCMET will achieve its goals by utilizing a wide variety of methodologies. The approach comprises computational tools for the prediction of biological activities and interactions, cell culture-based screening tools, up-to-date animal models, multi-layer omics technologies for the identification of molecular mechanisms, and also epidemiological data, in order to associate the exposure to chemicals to ED-related metabolic effects and to identify human biomarkers of exposure to metabolic EDs. Results from EDCMET are expected to substantially contribute to our mechanistic knowledge related to EDCs. Moreover, a tiered strategy for the testing of metabolic effects of EDs will be developed for future implementation in regulatory toxicology studies.

1902 Endocrine Disruptors Exert Cell Type-Specific Effects on Endogenous Glucocorticoid Signaling

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Endocrine disrupting chemicals (EDCs) are substances that interfere with functions of the endocrine system and can cause adverse developmental, neurological, metabolic, and reproductive effects. Unlike toxic effects that can be evident immediately, endocrine disruption may impact complex signaling pathways that take years or generations to discover. EDCs can affect several pathways in the body, but their potential effects on the glucocorticoid signaling is particularly important to study as the glucocorticoid receptor (GR) is present in almost every cell type and plays a critical role in development, metabolism, reproduction, and overall physiological homeostasis. The objective of this study was to evaluate the endocrine disrupting potential of a broad range of industrial chemicals, focusing on four chemicals with GR activity predicted by the Tox21 database: 4-nonylphenol (4-NP), bisphenol A (BPA), butylated hydroxytoluene (BHT), and phenolphthalein (PP). Exposure was tested in the human liver (HepG2) and uterine (Ishikawa) cell lines to understand potential impacts to metabolism and reproduction, respectively. We determined that these chemicals did not induce cell death at concentrations up to 1 uM, indicating that the dose range employed was not associated with toxicity. Instead, the chemicals tested altered the expression of classic glucocorticoid-responsive genes, *GILZ* and *PER1*. Interestingly, the effect was cell-type specific. For example, BHT significantly induced transcript levels of *GILZ* and *PER1* in HepG2 but not Ishikawa cells. We found that the induction of glucocorticoid-responsive genes by BHT was mediated by GR, as pretreatment with the GR antagonist RU 486 abolished the effect of BHT. Low-dose combinations of BPA and 4-NP or BHT produced additive effects on *GILZ* transcript levels in HepG2 cells, although the combination of BPA and 4-NP did not augment *GILZ* expression in Ishikawa cells. We found that co-treatment of chemicals with glucocorticoids altered the transcriptional response compared to glucocorticoids alone. To explore the mechanism of endocrine disruption, we evaluated the phosphorylation status of GR at two residues associated with GR activity: p211 and p226. The effect was specific to

cell type and chemical, where BPA and 4-NP enhanced p211 in Ishikawa but not in HepG2 cells. These results demonstrate that EDCs exert gene- and cell-type specific effects on glucocorticoid signaling.

1903 Comparative Phenotypic and Transcriptomic Responses to Multiple Endocrine-Disrupting Chemicals in Zebrafish

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Endocrine disrupting compounds (EDCs) are emerging contaminants of concern found ubiquitously in the environment that can cause a wide range of adverse effects, the full extent of which are currently unknown. We used zebrafish as a model to determine and compare the effects of nine EDCs (4-nonylphenol, atrazine, bisphenol-A, chlorpyrifos, dieldrin, estrone, metformin, triclocarban, and triclosan). Zebrafish are an NIH accepted model system for human health that have 70% homology with the human genome, transparent larvae (allowing observation of internal development), large numbers of offspring, and a sequenced genome. We exposed zebrafish to 3 concentrations of EDCs for either 1 or 5 days during early development and identified adverse developmental and behavioral outcomes. RNASeq and pathway analyses was performed to determine specific gene expression changes, as well as the critical pathways affected. Many differentially expressed genes overlapped across chemicals and some of the main pathways affected include reproductive diseases, endocrine system disorders, and estrogen synthesis and regulation. This study brings us closer to identifying the full extent of adverse effects of EDCs and the mechanisms through which these effects occur.

1904 Circadian Gene Expression in Rats is Altered by Repeated Exposure to Tetrabromobisphenol A

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The brominated flame retardant Tetrabromobisphenol A (TBBPA) is present in the epoxy resin of circuit boards used in electronic devices and has been found at significant levels in house dust. Studies performed by the National Toxicology Program have indicated a potential link between higher exposure levels and uterine tumors in rats, and TBBPA was listed as a probably carcinogen by the International Agency for Research on Cancer (IARC) in 2016. Evidence has also accumulated that TBBPA may have an endocrine disrupting function. In previous studies using Wistar Han rats chronically treated with 250 mg/kg/d for 5 days, our lab has linked TBBPA exposure to disruption in estrogen homeostasis, along with thyroid and immune system dysfunction. A potential effect on expression of the genes comprising the circadian clock was also discovered upon RNA-Seq analysis of liver and uterus from these TBBPA- and vehicle-treated rats. To investigate this effect, a circadian study was undertaken. Female Wistar Han rats were exposed to 250 mg/kg of TBBPA or vehicle for 5 consecutive days, then tissues were collected at 4-hour intervals and RNA was extracted. Expression levels of the core circadian genes Clock circadian regulator (*Clock*), aryl hydrocarbon nuclear translocator-like (*Arntl* or *Bmal1*), neuronal PAS domain protein 2 (*Npas2*), period circadian regulator 1, 2 and 3 (*Per1*, *Per2*, and *Per3)*, and cryptochrome circadian regulator 1 and 2 (*Cry1*, *Cry2*) and circadian-related genes retinoic acid receptor-related orphan receptor a, b and c (*Rora*, *Rorb*, *Rorc*), nuclear receptor subfamily 1 group D member 1 (*Nr1d1*, *Rev-erb*), casein kinase 1d (*Csnk1d*) and 1e (*Csnk1e*), aryl hydrocarbon receptor (*Ahr*), and aryl hydrocarbon receptor nuclear translocator (*Arnt*) were measured in liver and uterus using droplet digital PCR (ddPCR). Results showed differential effects in liver and uterus, including decreased amplitude, phase shift, and suppression of cyclicity, which appeared to be gene- and tissue-specific. These perturbations of the circadian cycle could affect expression of numerous genes which are reliant on circadian signals to regulate expression, including those controlling metabolism and the reproductive cycle. *This research was supported by the Intramural Research Program of NIH [Project ZIA BC 011476].*

1905 Towards Computational Modeling of Estrogen Receptor Alpha-Mediated Signaling in Endocrine Disruption

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Estrogen receptor alpha (ERα) belongs to the nuclear hormone receptor family of ligand-inducible transcription factors, and regulates gene networks in biological processes such as cell growth and proliferation. Disruption of these networks, for instance with the non-genotoxic carcinogen 17β-estradiol (E2), can result in adverse outcomes such as unanticipated cell proliferation ultimately culminating in tumor formation. Since ER signaling is also involved in normal physiological responses, and not solely activated in adverse outcomes, it is essential to quantify relationships between different key events leading to a particular adverse outcome induced by non-physiological ER activation. To obtain this quantitative information on these key events, a technique is favored which can provide single cell information on all these events. For this purpose, we established fluorescent protein reporter cell lines with bacterial artificial chromosome (BAC) green fluorescent protein (GFP) transgenomics of important players in the ERα signaling pathway in context of cellular proliferation. The human breast cancer cell line MCF7 was used as a model as its proliferation is dependent on ERα. In combination with advanced live cell imaging, these reporters can monitor the spatial and temporal dynamics of key events of ERα pathway activation, i.e. target activation and cell cycle progression, at a single cell level. This adverse outcome pathway-driven reporter platform allows us to quantify relationships between various different key events and the ultimate cellular adverse outcome, and to eventually integrate this dynamic signaling data in a computational model. In addition, these *in vitro* reporters can be used to screen e.g. drug candidates or other chemicals of concern for the potential of modulating ER activity and the likelihood of a non-genotoxic carcinogenic mode of action.

1906 Development of Strategies to Determine the *In Vitro* and *Ex Vivo* Induction of T_4-glucuronidation in Different Species

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The thyroid hormones (THs) triiodothyroxine (T_3) and thyroxine (T_4) are essential for normal cellular differentiation and growth. The prohormone T_4 can be converted enzymatically into the biologically active T_3 and as such functions as a reservoir for T_3. Xenobiotics can alter the homeostasis of THs through induction of Phase I and Phase II enzymes resulting in increased metabolism. One of the metabolic pathways by which xenobiotics may cause effects on TH homeostasis is induction of hepatic glucuronidation. Both T_3 and T_4 can be glucuronidated and the formed glucuronides can no longer be converted into active T_3 and are biliary excreted. As a consequence, T_3 and T_4 plasma levels may decrease resulting in hypothyroidism which may cause adverse health effects. In the current study, strategies to determine induction of T_4-glucuronidation in different species (mouse, rat, dog and human) were evaluated. First, UPLC-PDA-MS-based analytical methods to monitor T_4 and its metabolites were developed and implemented. To investigate if T_4 metabolism is species- and/or gender-specific, T_4-glucuronidation was determined using male and female liver microsomes from all four species. Subsequently, the metabolism of T_4 was also studied in male and female hepatocytes from all four species during which not only the formation of T_4-glucuronide but also formation of T_4-sulphate, T_3 and 3,3',5'-triiodothyronine (rT_3) was investigated. For *in vitro* induction, the effect of inducers upon the T_4 metabolism was studied using rat and human hepatocytes and conditions were optimized to develop an assay that can be used to determine xenobiotic-induced T_4 metabolism. For the *ex vivo* induction, protein- and time-dependent experiments were performed for selected species to optimize reaction conditions for *ex vivo* experiments. Experiments were also performed to evaluate the activity of commercially available xenobiotic-induced rat liver microsomal fractions. Overall, the current study gives an overview of species-, gender- and inducer-specific differences for the metabolism of T_4. The here presented assays can be used to determine the *in vitro* and *ex vivo* induction of T_4-glucuronidation in different species.

1907 Multi-Endocrine Disruptors Screening in Zebrafish

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In vitro tools are inexpensive and scalable for high-throughput platforms, however they pose low relevance to vertebrate species. On the other hand, work with adult animals is expensive and represent ethical issues. Small fish like zebrafish (*Danio rerio*) are excellent alternative to *in vitro* and *in vivo* model. They offer a unique experimental system where screening assays can be performed at the whole animal level, being at the same time compatible with the 3R principles (replacement, reduction and refinement in animal testing). Endocrine disruptors (EDCs) are chemicals that by interfering with the endocrine system can have an adverse effect at developmental, neurological, immune and reproductive level. Thyroid Disrupting (TD) compounds specifically alter the function of thyroid gland through the interference with the synthesis, transport and/or binding of the thyroid hormones. The negative impact of EDCs is becoming a real public health issue, therefore the necessity of tests to assess the potential risk of new chemicals before they are marketed is increasing. The zebrafish is currently used as a model for the evaluation of acute and developmental toxicity and for the screening and testing of potential thyroid (TD) and endocrine disrupters (EDCs), as described in the OECD Guidelines. The two major endpoints used to evaluate EDCs, vitellogenin concentration and change in sex ratio, have several limitations. With the purpose of expanding the number of tests available to identify estrogenic and androgenic substance, we evaluate gene expression of 4 biomarkers in 5 dpf zebrafish larvae after exposure, from 48 hpf, to 10 compounds reported as EDCs. Expression assay over known markers of thyroid pathway was also developed to evaluate 9 environmentally relevant TD substances. This screening methodology showed to be a sensitive and cost-effective assay to screen and evaluate potential EDCs chemicals.

1908 The Intervention of NLRP3 Inhibitor and Nrf2 Activator Protects against Diethyl Nitrosamine and Thioacetamide-Induced Hepatic Precarcinogenesis in Rats

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More than 30% of all malignancies initiated/exacerbated by inflammation and support inflammation as a pivotal component of tumor initiation. Nrf2 plays complex and multicellular roles in hepatic inflammation, carcinoma, fibrosis, and regeneration. Hepatocellular carcinoma (HCC) is the second leading cause of cancer mortality worldwide. Thioacetamide (TAA), a hepatotoxicant precisely mimics the characteristics of human liver damage in animal models and induces liver fibrosis, cirrhosis, and carcinoma. Diethyl nitrosamine (DEN) is a carcinogen, promotes carcinogenesis. Antidiabetic drug glibenclamide (GLB) possesses anti-inflammatory properties and inhibits NLRP3 inflammasome. Dimethyl fumarate (DMF), a multiple sclerosis drug, activates the Nrf2/ARE pathway and maintains the antioxidant status. The aim is to investigate (i) chronic inflammatory condition that affects liver regeneration (ii) the chemo-protective effect of NLRP3 inhibitor glibenclamide and Nrf2 activator dimethyl fumarate on DEN+TAA-induced hepatic precarcinogenesis, in partial hepatectomized Wistar rats. Rats were subjected once to DEN and after two weeks to partial hepatectomy. TAA (100 mg/kg every third day, ip), DMF and GLB (DMF 25 mg/kg/day, po and GLB 0.5 mg/kg/po) were administered for 16 weeks. GLB and DMF intervention improved the DEN+TAA induced reduction in body weight, increased liver weight, and plasma transaminase levels. GLB and DMF treatment improved DEN+TAA-induced foci of altered hepatocytes, fatty degeneration, multifocal steatosis, lymphocyte infiltration, collagen deposition, necrosis, DNA damage, and apoptosis. Further, GLB and DMF treatment significantly restored the DEN+TAA-induced alterations in inflammatory (NLRP3, ASC, caspase1, NF-κB), fibrogenic (TGFβ1, collagen), anti-oxidant (Nrf2, HO-1, SOD1, catalase), and regenerative proliferative stress (PCNA, pSTAT3/STAT3, GST-p, HGF, cMET, TGFα, EGF, AFP) markers. Hepatoprotective responses of GLB and DMF were evident by the restoration of DEN+TAA-induced oxidative stress, inflammatory responses, DNA damage, histopathological changes, and anti-oxidant levels. Simultaneous maintenance of antioxidant status by activation of Nrf2 and reduction of the inflammatory condition by the inhibition of NLRP3 could be a rational strategy for improving liver regeneration and to reduce the development of HCC.

1909 Gene Expression Signature of Dieldrin-Treated Livers in Mice

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Dieldrin, an organochlorine insecticide, induced liver tumors in mice after chronic exposure. Although no longer manufactured, dieldrin is still present in the environment and in human tissues. Previous work in our lab showed a defined dose response effect of dieldrin on liver cell proliferation and oxidative stress induction suggesting a role of oxidative stress as the mode of action for the observed murine liver tumors. Subsequently as our understanding of nuclear receptor mediated processes in liver carcinogenesis has advanced, we examined a role for nuclear receptor activation in dieldrin induced cancer. The current studies showed CAR (constitutive androstane receptor) activation in dieldrin treated mice. We also examined, using RNA sequencing in both C57BL/6 and B6C3F1 mouse livers, the effect of dieldrin (10 ppm in diet for 14 days). In B6C3F1 mice, there was a significant increase in the expression of genes specific to CAR activation (*Cyp2b6, Cyp2c8, Cyp2c9, Cyp2b6)*. A decrease in *Cyp27b1* was noted that suggests a negative vitamin D receptor response. PXR/RXR activation, VDR/RXR activation, and aryl hydrocarbon receptor signaling pathway were also altered. Pathway analysis showed hepatocellular carcinoma pathways were upregulated. Dieldrin treated C57BL/6 mice showed a similar Cyp2c family induction (CAR activation) however no significant alteration of the PXR/RXR pathway was seen. Instead, insulin-dependent diabetes mellitus, diabetes mellitus, and glucose metabolism disorder pathways were downregulated. LPS/IL-1 mediated inhibition of RXR function pathways was altered in both species, and also estrogen biosynthesis pathways was upregulated. Estrogen was shown to be an effective activator of CAR in both female and male mice in the past, supporting dieldrin as a CAR activator. The CAR activation pattern seen with dieldrin mimics that seen with other organochlorine insecticides including toxaphene thus pointing to the mode of action of this group of compounds as CAR mediated mode of action for the observed rodent liver tumors.

1910 NR2E3 Is a Key Component in p53 Activation by Regulating a Long Noncoding RNA DinO in Acute Liver Injuries

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Damage-induced long noncoding RNA (DINO) is a long noncoding RNA that directly interacts with p53 and thereby enhances p53 stability and activity in response to various cellular stresses. Here, we demonstrate that nuclear receptor subfamily 2 group E member 3 (NR2E3) plays a crucial role in maintaining active DINO epigenetic status for its proper induction and subsequent p53 activation. In acetaminophen (APAP)- or carbon tetrachloride-induced acute liver injuries, NR2E3 knockout (KO) mice exhibited far more severe liver injuries due to impaired DINO induction and p53 activation. Mechanistically, NR2E3 loss both *in vivo* and *in vitro* induced epigenetic DINO repression accompanied by reduced DINO chromatin accessibility. Furthermore, compared with the efficient reversal by a typical antidote N-acetylcysteine (NAC) treatment of APAP-induced liver injury in wild-type mice, the liver injury of NR2E3 KO mice was not effectively reversed, indicating that an intact NR2E3-DINO-p53-signaling axis is essential for NAC-mediated recovery against APAP-induced hepatotoxicity. These findings establish that NR2E3 is a critical component in p53 activation and a novel susceptibility factor to drug- or toxicant-induced acute liver injuries.

1911 Control Background Data Collection for Conducting General Toxicity Studies Using PXB-Mice

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PXB-mice, immuno-deficient chimeric mice with humanized liver, have mostly been used in hepatitis-related efficacy studies. Recently, the number of PK and general toxicity studies using this model has increased, and the trend is expected to continue in the future. However, the background data of this model is limited; therefore, we collected data for examinations commonly conducted in general toxicity studies and compared them with male mice from other strains. In male PXB-mice housed at SNBL between 2018 and 2019, clinical signs (n=192, 14-49 weeks old), body weight (n=192, 14-21 weeks old), food consumption (n=16, 14-17 weeks old), ophthalmology (n=17, 28-44

weeks old), hematology, blood chemistry (n=21, 15-21 weeks old), necropsy (n=45, 15-49 weeks old), liver weight (n=23, 15-49 weeks old, liver only), and histopathology (n=6, 23-24 weeks old) data were collected and compared with our background data on male ICR (CD1), C57BL/J, and/or NOG mice. Human-ALT and human-albumin in PXB-mice plasma were also measured. Only the results that are specific to features of PXB-mice are described In clinical signs, of 192 animals, 24 animals (12%) were found dead or euthanized due to moribundity at 19-32 weeks old, and abdominal distension was observed in 15 of these 24 animals (62%). At necropsy of dead or euthanized animals, enlargement of the liver, spleen, and thymus and/or a black focus in the brain were observed in 8 animals. Low values in body weight and hematocrit (HCT), MCV, and MCH in hematology and high values in AST, ALT, ALP, potassium, total protein, albumin, and globulin in blood chemistry were noted. Human-ALT ranged from 5.8 to 24 ng/mL and human-albumin from 0.9 to 1.7 g/dL. In ophthalmology, corneal opacity was observed in 6 of 17 animals (35%), and this was accompanied by angiogenesis in 1 animal. At necropsy, liver weight of all animals was over 2-fold that in other strains. In histopathology, hypoplasia in the lymphoid organs and vacuolation in the hepatocytes were observed in all animals. Lymphoma was observed in 1 of 6 animals. Based on the above, many differences, mainly variations in liver related parameters and histopathological findings in immune-related organs, were observed in PXB-mice. The above mentioned information should be taken into consideration for toxicity evaluation when using PXB-mice as an animal model.

1912 Exogenous Phosphatidic Acid Is a Novel and Widely Available Treatment for Acetaminophen Hepatotoxicity

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Acetaminophen (APAP) overdose causes severe liver injury through a mechanism involving metabolism, oxidative stress, and mitochondrial damage, while autophagy and regulatory Kupffer cells (KCs) are protective. We recently discovered that endogenous phosphatidic acid (PA) is increased in the liver after APAP overdose and enhances liver regeneration. PA is also available over-the-counter as a dietary supplement. Thus, we hypothesized that exogenous PA could be used as a readily available treatment for APAP hepatotoxicity. Mice were treated with APAP (250 mg/kg i.p.) at 0h, followed by either PA (20 mg/kg i.p.) or vehicle (i.p.) at 2, 6, 24, and 48h. Blood and liver tissue were harvested at 6, 24, and 52h. Some mice were pre-treated with liposomal clodronate to deplete KCs. APAP metabolism was assessed by liver glutathione and APAP-protein binding. Oxidative stress was assessed by oxidized glutathione. Mitochondrial damage was assessed by mitochondrial protein release. Other parameters were measured by immunoblotting. PA treatment reduced liver injury (serum ALT, histology), but did not affect regeneration (PCNA immunoblot). Surprisingly, there was no difference in APAP metabolism, oxidative stress, mitochondrial damage, JNK activation, or autophagy between Veh and PA-treated mice. Proteomics indicated the involvement of KCs. However, depletion of KCs did not affect the protection by PA. PA is a novel treatment for APAP hepatotoxicity that is immediately available. Exogenous PA treatment protects against acetaminophen toxicity through a novel mechanism. Further exploration of the mechanism may bring new insight into the well-studied pathophysiology of APAP-induced liver injury.

1913 The Role of Secretory Phospholipase A2 (sPLA2) in Mediating the Progression of Injury in Acetaminophen-Induced Acute Liver Failure

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Acetaminophen (APAP) overdose is the leading cause of acute liver failure (ALF) in the United States with limited treatment options. APAP induced hepatotoxicity continues to progress even after the initial toxicant APAP has been eliminated from the body. Following APAP overdose, toxicity is initiated by conversion of APAP to its reactive metabolite N-acetyl-p-benzoquinone (NAPQI), which binds irreversibly to form NAPQI-protein adducts. This irreversible binding of NAPQI-protein adducts leads to hepatocellular necrosis. The focus of this study is to investigate the role of APAP induced ALF through the leakage of cytosolic enzymes and their interaction in recruitment of pro-inflammatory cytokines to the site of injury. We hypothesize that sPLA2 will mediate the progression of APAP induced ALF through the modulation of pro-inflammatory cytokines. Preliminary data from *in vivo* mice studies indicate that following injury there is higher levels of sPLA2 released in the extracellular matrix. We have also observed higher levels of pro-inflammatory cytokines in APAP overdose C57/BL6J mice. However, the mechanism by which the increased sPLA2 contributes to the progression of injury is not clear. Planned experiments aim to investigate the mechanism via which sPLA2 mediates progression of liver injury and whether induced sPLA2 modulates prostaglandins and cytokine pathways to mediate progression of injury. To test our hypothesis, we will dose the HepG2 cells with varying APAP. After treating the cells in a dose dependent manner, we will perform cytotoxicity assay over time course and quantify the cell viability. Then we will quantify the sPLA2 activity in these different experimental conditions from the collected supernatant. We will also test whether addition of sPLA2 to healthy hepatocytes leads to disruption of cell membrane integrity and necrosis. We expect that hepatotoxicity leads to increased sPLA2 activity, and the induced sPLA2 will continue the progression of damage in healthy hepatocytes even in absence of APAP. Via this approach, we can find a novel therapeutic approach to prevent the progression of injury following APAP overdose.

1914 Mode-of-Action Studies for Flupyrsulfuron Methyl Demonstrate Nonhuman Relevance of Mouse Liver Tumors

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In an 18-month mouse study, increased incidences of hepatocellular adenomas were observed in males and females administered diet containing 2500 or 7000 ppm flupyrsulfuron methyl (FLP). Mode of action studies were conducted to determine if CAR/PXR nuclear receptor activation could be responsible for increased tumor incidence. In a 28-day feeding study to evaluate liver mechanistic parameters, FLP was administered to male and female mice at dietary concentrations of 0, 250, 2500, and 7000 ppm. Parameters included body weight, food consumption, clinical signs, liver weight, hepatic histopathology and cell proliferation, gene expression and enzyme activity for nuclear receptor mediated cytochrome P450. Additional groups were dosed with 1000 ppm of phenobarbital (PB), the positive control for CAR/PXR activation. In a second study, FLP was evaluated for the induction of replicative DNA synthesis (RDS) and expression of *CYP2B* and *CYP3A* in mouse and human hepatocytes *in vitro*. The RDS and gene expression assays were conducted as separate assays, and a corresponding ATP assay was conducted for each to assess the toxicity of FLP. The hepatocyte cultures were exposed for 96 hours to 0.16, 0.8, 4, 20, 100, 500, and 1000 µM FLP or to 100, 500, and 1000 µM PB. The positive controls epidermal growth factor (50 ng/mL- mouse) and hepatic growth factor (100 ng/mL- human) were used in the RDS assay. Exposure of mice to FLP resulted in increased liver weights, hepatocellular hypertrophy, and increased hepatocellular proliferation associated with an increase in mitotic figures at 2500 and 7000 ppm. These findings were of weaker magnitude compared to those observed in 1000 ppm PB mice. Liver changes occurred in association with induction of cytochrome P450 enzyme activities and gene expression consistent with the response seen with PB, albeit much weaker in pattern. In the *in vitro* hepatocyte cultures, FLP induced a statistically significant RDS response in mouse hepatocytes that was highest at 4 or 20 µM due to cytotoxicity at higher concentrations. *Cyp2b10* gene expression was induced at 1000 µM and *Cyp3a11* was induced at 500 and 1000 µM. FLP did not induce an RDS response in human hepatocytes at any concentration evaluated. FLP induced *CYP2B6* and *CYP3A4* in human hepatocytes at 100 µM. Cytotoxicity occurred at higher concentrations. The positive controls also fulfilled the requirements of a valid test. These data support the conclusion that the liver responses to FLP are modulated *via* the CAR nuclear receptor, a mechanism not relevant for tumor induction in humans.

1916 Hepatotoxicity, Lipid Peroxidation, and Perturbation in Antioxidant Defense System Induced by Emamectin Benzoate and Acetamiprid Combination in Male Rats

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Pesticides are widely used in agriculture to maintain and increase crop yields, in addition to their use in homes and gardens. Exposure to pesticides is one of the major environmental health problems due to their potential adverse effects on non-target organisms. So, the current study aims to investigate the effect of emamectin benzoate and acetamiprid alone and in combination on lipid peroxidation, enzymatic and non-enzymatic antioxidants, liver functions biomarkers in male rats in addition to histological and immunohistochemical examination. Male Wister albino rats were divided randomly into four groups of seven each, group I used as control; group II treated with emamectin benzoate (EMB; 2.5 mg / kg BW), group III received acetamiprid (ACM; 30 mg/kg BW) and group IV treated with both EMB and ACM, respectively. Rats were

orally administered their respective doses every day for three weeks. The administration of EMB, ACM alone or in combination significantly increased lipid peroxidation (LPO) and reduced the activities of antioxidant enzymes (SOD, CAT, GPx, GR, GST), alkaline phosphatase (ALP), aminotransferases (AST, ALT) in addition to reduced glutathione and protein contents. While, lactate dehydrogenase (LDH) activity was significantly elevated. Histopathological and PCNA-immunoreactivity examinations confirmed the biochemical results and supported the detrimental effect of insecticides. Conclusively, it is clear that EMB and ACM induced pronounced hazardous effects in rat liver especially in the combination group where a synergistic effect was observed.

1917 Mitochondrial Morphology and Functional Alterations in Acetaminophen Hepatotoxicity

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Generation of reactive oxygen species (ROS) and mitochondrial oxidant stress are hallmarks of acetaminophen (APAP) overdose, however, the temporal relationship between changes in mitochondrial function and morphology during APAP-induced hepatocyte necrosis has not been delineated. This is important since the crosstalk between mitochondrial morphology and organelle function is now recognized to modulate disease pathophysiology, and we have earlier demonstrated mitochondrial fragmentation after an APAP overdose. To determine the relationship between this change in morphology and mitochondrial function, we isolated mouse primary hepatocytes from 8-week-old C57Bl/6J mice and treated the cells with varying concentrations of APAP (1-10mM). Cells were then stained with Mitotracker Green to visualize mitochondrial morphology changes over time. Initially, isolated hepatocytes display a diverse array of elongated and rounded mitochondria forming a complex network throughout the cell. However, hepatocytes treated with 10 mM APAP undergo a dramatic change in mitochondrial morphology at 4 hr whereby many mitochondria become small and rounded with a "donut" appearance. These morphological changes correspond with a decrease in basal respiration, maximal respiratory capacity, and spare respiratory capacity which occurs prior to overt mitochondrial depolarization. By 9 hr hepatocytes treated with 10 mM APAP exhibit nearly ubiquitous round, donut mitochondria with elongated mitochondria being absent from most of the cells. This morphological phenotype corresponds with a further decrease in respiratory function and mitochondrial membrane potential. These morphological effects seem to be concentration dependent since hepatocytes treated with 1 mM or 2 mM APAP do not exhibit a change in mitochondrial shape. Interestingly, treatment with 5 mM APAP elicits a delayed change in mitochondria morphology, where the donut shaped mitochondria appear ubiquitously by 9 hr. Collectively, this data highlights the complex interplay between mitochondrial morphology and function during APAP-induced hepatocyte toxicity.

1918 Fibrinogen Engagement of Leukocyte β_2 Integrins Balances Monocyte/Neutrophil Accumulation to Inhibit Acetaminophen-Induced Liver Injury

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Leukocytes play a multifactorial role in the injured liver following acetaminophen (APAP) overdose. Specific subsets of leukocytes exacerbate hepatotoxicity while others mediate liver repair. The balance of these activities is controlled in part by the blood clotting protein fibrin(ogen). Prior studies document that fibrin(ogen) engagement of leukocyte β_2 integrins inhibits liver necrosis and promotes repair after APAP overdose. Whether that outcome is a product of altered leukocyte recruitment or impaired local effector function remains unknown. We hypothesized that monocytes recruited to the injured liver are driven to a pro-repair phenotype by fibrinogen-β_2 integrin engagement. Male mice expressing a mutant fibrinogen incapable of engaging β_2 integrins (Fib$\gamma^{390-396a}$ mice) and wild-type mice were challenged with a hepatotoxic dose of APAP (300 mg/kg, i.p.). Hepatic leukocyte populations were quantified by flow cytometry 24 hours later. As expected, hepatic necrosis was increased in APAP-challenged Fib$\gamma^{390-396a}$ mice compared to APAP-challenged wild-type mice. In wild-type mice, there was a dramatic accumulation of monocytes (CD11b+/Ly6G-/Ly6C+) in the injured liver, comprising ~28% of total immune cells compared to ~3% in vehicle-treated mice. Interestingly, hepatic monocytes were reduced in livers of APAP-challenged Fib$\gamma^{390-396a}$ mice. The percentage of monocyte-derived macrophages (CD11b+/Ly6G-/Ly6C-low/F480+/CX3CR1+), which have a pro-repair function in the APAP-injured liver, was also reduced in APAP-challenged Fib$\gamma^{390-396a}$ mice. Unexpectedly, we observed a dramatic increase in hepatic neutrophils (CD11b+/Ly6G+) in APAP-challenged Fib$\gamma^{390-396a}$ mice. Analysis of whole-liver RNA sequencing revealed an imbalance in hepatic chemokine expression, confirmed by qRT-PCR, including diminished induction of monocyte chemokine *Ccl2* and exaggerated expression of the neutrophil chemokine *Cxcl2* in APAP-challenged Fib$\gamma^{390-396a}$ mice. The results indicate that fibrin(ogen)-β_2 integrin engagement plays a critical role in regulating chemokine expression driving leukocyte accumulation in the APAP-injured liver. The absence of fibrin(ogen)-β_2 integrin engagement causes imbalanced leukocyte accumulation disabling liver repair and exacerbating hepatotoxicity.

1919 Omega 3- Fatty Acids and Vitamin E Protect against Diazinon-Induced Oxidative Injury and Biochemical Perturbations in Rat Liver

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This study was designed to investigate the possibility of diazinon to induce oxidative stress and biochemical changes in rat liver and the role of Omega 3- fatty acids and Vitamin E in alleviating their toxic effects. Male Wister albino rats were divided randomly into six groups of seven each, group I used as control; group II treated with omega 3- fatty acid (ω3FA; 200 µg/kg BW), group III received vitamin E (VE; 100 mg/kg BW), group IV treated diazinon (DZN; 15 mg/kg BW), group V treated with both ω3FA and DZN while group VI was treated with VE plus DZN. Rats were orally administered their respective doses daily for 30 days. Results revealed that the administration of DZN caused elevation in lipid peroxidation (LPO) and reduction in enzymatic antioxidant activities including catalase (CAT), superoxide dismutase (SOD), glutathione *S*-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR). Also, a decrease in reduced glutathione (GSH) content were also observed. Liver aminotransferases (AST and ALT) and alkaline phosphatase (ALP) were decreased, while lactate dehydrogenase (LDH) was increased. Conclusively, it is clear that DZN -induced oxidative damage in rat liver while ω3FA and VE had powerful effects to protect against DZN induced hepatotoxicity and modulated most of the studied parameters near normal level, and the effect of ω3FA is more pronounced than VE.

1920 Ochratoxin A Induces Hepatic Fibrosis through TGF-β/Smad2/3 Signaling Pathway

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Ochratoxin A (OTA) is a mycotoxin produced by species of *Aspergillus* and *Penicillium*. It is often formed in cereals under high temperatures and humid conditions. In this study, we assumed that OTA causes epithelial-mesenchymal transition (EMT) leading to liver fibrosis. In this study, we investigated that the TGF-β1/Smad2/3 signaling pathway is involved in EMT-induced liver fibrosis. In our *in vitro* and *in vivo* experiments, the mRNA and protein expression of major liver fibrosis markers such as fibronectin, α-SMA, and E-cadherin were measured. Additionally, ALP, ALT, AST, and bilirubin for liver damage were measured. We confirmed the increased mRNA and protein expression of TGF-β1, Smad2, and Smad3. After the expression of Smad2 and Smad3 was knockdown by siRNA, the expression of the liver fibrosis-related markers was inhibited. The liver cells exposed to OTA enhanced TGF-β1 receptor expression on the cell membrane. Then, in the cytoplasm, Smad2 and Smad3 are phosphorylated and translocated to the nucleus, which could contribute to fibrosis due to EMT. These results can provide the basis for the treatment and prevention against OTA-induced hepatic fibrosis.

1921 Late Protective Effect of Netrin-1 in the Murine Acetaminophen Hepatotoxicity Model

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Acetaminophen (APAP) hepatotoxicity is the leading cause of acute liver failure in the United States and the current antidote for APAP overdose is administration of N-acetylcysteine (NAC), which facilitates scavenging of the reactive APAP-metabolite NAPQI and reactive oxygen species. However, NAC has a short early therapeutic window and novel therapeutic intervention strategies are needed for patients who typically present late to the hospital. This is relevant, since the recovery and regeneration capacity of the liver, which typically occurs late after injury, plays a critical role in patient survival after an APAP overdose. Netrin-1 is a laminin-related protein and recent work has

demonstrated that netrin-1 promotes hepatic repair and regeneration during liver ischemia/reperfusion injury. The role of Netrin-1 in acetaminophen hepatotoxicity is unknown and this study evaluated this *in vivo* in a mouse model of APAP overdose. Male C57BL/6J mice were co-treated with exogenous netrin-1 or vehicle control, along with 300mg/kg APAP and euthanized at 6h and 24h. While netrin-1 administration did not influence ALT levels or area of necrosis at the 6h time point. However by 24h, netrin-1 treated animals showed significant attenuation of liver injury as seen by ALT levels and area of necrosis. This protection was accompanied by significant elevations in mRNA and protein level of the adenosine A2B receptor (A2BAR), one of the netrin-1 receptors. Exogenous netrin-1 also promoted infiltration of F4/80 positive macrophages toward areas of necrosis and promoted liver regeneration by 24h. Treatment with the A2BAR antagonist PSB 1115 negated the protective effects of netrin-1, including the enhanced macrophage infiltration and liver regeneration indicating that netrin-1 protection was mediated through the A2BAR. Removal of resident Kupffer cells with clodronate liposomes also prevented the netrin-1 induced increase in liver macrophages and liver regeneration and removed protection against liver injury. In conclusion, our data indicate a previously unrecognized role for netrin-1 in attenuation of APAP hepatotoxicity, which could be mediated by promoting macrophage migration and liver regeneration through the A2BAR.

1922 Mitochondrial Protein Adduct and Superoxide Generation Are Prerequisites for Early Activation of c-Jun N-terminal Kinase within the Cytosol after an Acetaminophen Overdose in Mice

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Acetaminophen (APAP) overdose is the most common cause of acute liver failure in the United States and formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) is critical for initiating APAP-induced liver injury. Though formation of mitochondrial NAPQI-protein adducts, mitochondrial oxidant stress and activation of the MAP kinase c-jun N-terminal kinase (JNK) have been shown to be critical for APAP-induced cell death, direct evidence for a requirement of mitochondrial adduct formation for the initiation of JNK activation in the cytosol is lacking. We examined mitochondrial protein adduct levels by measuring protein-derived APAP-cysteine by HPLC and JNK activation by Western blotting at very early time points after treatment with 300 mg/kg APAP. The role of oxidant stress in this process was assessed by *in vitro* and *in vivo* experiments. Measurement of mitochondrial protein adducts in C57Bl/6J mice treated with 300mg/kg APAP indicated detectable levels of protein adducts (0.05-0.1 nmol/mg protein) by 15 and 30 min after APAP. After >90% depletion of hepatic GSH levels at 30 min, there was a selective increase (>500%) of mitochondrial protein adducts between 30 and 60 min. However, activation of the MAP kinase JNK in the cytosol was only evident at 60 min, suggesting that mitochondrial protein adduct formation was required for the initial JNK activation. To further confirm the role of oxidant stress exclusively within the mitochondria for cytosolic JNK activation, experiments were repeated after a 1h pre-treatment of mice with either Mito-TEMPO (which scavenges superoxide within mitochondria) or TEMPO (which scavenges superoxide in the cytosol only). Interestingly, both treatments showed significant attenuation of JNK phosphorylation in the cytosol at 60 min after APAP. *In vitro* experiments using primary mouse hepatocytes treated with 10mM APAP showed early superoxide formation from mitochondria by 30 minutes. Our studies provide direct evidence that the early mitochondrial superoxide formation caused by NAPQI-protein adducts within the organelle is a critical requirement for activation of the MAP kinase cascade resulting in the initial JNK phosphorylation in the cytosol and translocation to the mitochondria.

1923 Activation of p62-keap1-Nrf2 Antioxidant Pathway by Rutaecarpine in the Early Stage of Acetaminophen-Induced Acute Liver Injury in Mice

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Rutaecarpine, an indolopyridoquinazolinone alkaloid isolated from the unripe fruit of *Evodia rutaecarpa*, have been used the abdominal pain, amenorrhea, dysentery, headache, hypertension, and postpartum hemorrhage for traditional oriental medicine in East Asia. However, the inhibitory effect of rutaecarpine on p62-keap1-Nrf2 antioxidant pathway in acetaminophen (APAP)-induced hepatotoxicity remains unknown. This study investigated the regulatory effect of rutaecarpine action to attenuate acetaminophen-induced hepatotoxicity in mice. Rutaecarpine significantly decreased the APAP-induced serum ALT/AST activities and hepatic malondialdehyde content, while rutaecarpine prevented hepatic glutathione depletion by APAP. Furthermore, CYP2E1 expression was decreased by rutaecarpine treatment in a dose-dependent manner. Rutaecarpine treatment attenuated the APAP-induced inflammatory cytokines mRNA expression and production through inhibition of NF-κB activation. Also, rutaecarpine promoted activation of Nrf2-mediated target anti-oxidant enzymes, GCLC, HO-1, and NQO1, via APAP-induced Keap1 degradation. Interestingly, rutaecarpine treatment increased the APAP-induced elevation of LC3 I to LC3 II and the degradation of p62, which attenuated acetaminophen-induced JNK1/2 activation. These findings proved perspectives that protective effect of rutaecarpine on acetaminophen-induced acute liver injuries via activation of antioxidant enzymes and autophagy. Therefore, rutaecarpine could be a useful therapeutic agent for the discovery of new chemotherapeutic agents that may contribute to protecting the liver injury by hepatotoxicants.

1924 Effect of the Organophosphorus Pesticide Temephos on Hepatic Function

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Temephos (Tem) is the larvicide of choice to control virus-transmitting vectors, such as dengue, zika, and chikungunya. Tem is considered a low toxicity pesticide by acute exposures. The toxicological information about Tem is limited. The aim of this study was to evaluate the effect of repeated doses of Tem on liver function and its metabolism. Male adult Wistar rats were orally administered with Tem at the dose of 100 mg/kg/d for 7 d emulsified in saline solution and euthanized 1-hour after the last dose. Blood was obtained by cardiac puncture and the liver was removed. Serum was used for the determination of bilirubin, enzyme activities of alanine aminotransferase (ALT), aspartate transaminase (AST), and γ-glutamyl transpeptidase (γ-GTP), and for the analysis of Tem and its metabolites by HPLC-DAD. Tem exposure resulted in a decrease in the weight of the animals from the fourth dose, which led to the death of 41% of the animals, as well as a significant decrease in the relative weight of the liver and serum levels of total lipids, triglycerides, and very low-density cholesterol. Conversely, serum levels of direct, indirect, and total bilirubin significantly increased. At least eight metabolites in extracts of liver were detected, among them Tem-sulfoxide, Tem-oxon, Tem-oxon-sulfoxide, Tem-dioxon-sulfone, Tem-oxon-sulfoxide-mono-hydrolyzed, 4,4´-thiodiphenol, 4,4´-sulfinyldiphenol, and 4,4´-sulfonyldiphenol or bisphenol S (BPS). No significant effect on hepatic total cytochrome P450 levels was observed but in an *in vitro* enzymatic assay using Tem-treated rat liver microsomes, a different profile of Tem metabolites was observed, compared to control microsomes, which indicates that Tem affects its own metabolism. Serum enzyme activities of ALT, AST, and γ-GTP were not altered. These results suggest that the liver is a target organ of Tem since it alters the balance of lipids and the metabolism of endogenous and exogenous compounds. In addition, these data contribute to the toxicological characterization of this pesticide widely used in tropical countries.

1925 Evaluation of Hepatic and Renal Effects of Acute and Subacute Oral Exposure to Bromochloromethane (BCM) in Rats

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BCM is a volatile organic compound that has been used as a fire extinguishing agent as well as an intermediate in the production of solvents. BCM is on EPA's Candidate Contaminant List 4 and is identified as a water disinfection byproduct (DBP). It is a structural analogue to chloroform (CHCl3) and bromodichloromethane (BDCM), DBPs that have been shown to be hepatotoxic and/or nephrotoxic in animal studies. There are limited data on the hepatic and renal effects associated with acute and subacute exposure to BCM. In the current study, male and female Fischer 344 rats received a single dose of 1000 mg/kg body weight BCM by oral gavage in 10% Alkamuls aqueous vehicle at 5 ml/kg gavage volume to assess toxicity over 48 hours. Additional sets of each gender were gavaged daily for 10 consecutive days to assess hepatic and renal effects. A single 1000 mg/kg BCM exposure did not affect body, relative liver and kidney weights in either gender at 24 or 48 hr post exposure. Serum markers of liver damage were not affected at 24 hr in either gender; however, by 48 hr glucose, albumin and total protein were significantly decreased in males. No liver histological damage was evident in either gender at 24 or 48 hr. In females, urinary markers of kidney damage, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and aspartate aminotransferase were slightly but significantly increased at earlier time points but

had returned to control values by 48 hr. ALP and GGT changes were sustained over 48 hr in males. Subacute exposure at 1000 mg/kg BCM resulted in unexpected (reported oral LD50 = 5000 mg/kg) high mortality in both genders with the study terminated by day 3. Exposure to 750 mg/kg BCM daily for 10 consecutive days did not affect body, relative liver and kidney weights in either gender. No significant increases in serum markers of liver damage were apparent in either gender which was confirmed histologically. While urinary protein increased significantly in females and disrupted the diurnal cycle of GGT observed in control male rats, subacute exposure to BCM did not result in histologic renal damage. This study indicates that BCM is less overtly toxic to rat liver and kidney than its structural analogues CHCl3 and BDCM. *This abstract does not reflect US EPA policy.*

1926 Deletion of Hepatic Yap Impairs Car-Driven Hepatocyte Proliferation without Altering Induction of Drug Metabolism Genes in Mice

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Constitutive androstane receptor (CAR) is known to be activated by several clinically used drugs, environmental pollutants and other exogenous compounds. CAR agonists, such as TCPOBOP, are known to cause robust hepatocyte proliferation and hepatomegaly in mice along with induction of drug metabolism genes, without any associated liver injury. Yes-associated protein (YAP) is a key transcription regulator that tightly controls organ size including that of liver. Ours and other previous studies suggested increased nuclear localization and activation of YAP after TCPOBOP treatment in mice and potential role of YAP in CAR-driven proliferative response. Here, we investigated a direct role of YAP in CAR-driven hepatomegaly and hepatocyte proliferation using hepatocyte-specific YAP KO mice. AAV8-TBG-CRE vector was injected to YAP-floxed mice for achieving hepatocyte-specific YAP deletion followed by TCPOBOP treatment. YAP deletion did not alter protein expression of CAR or CAR-driven induction of drug metabolism genes (including Cyp2b10, Cyp2c55 and UGT1a1). However, YAP deletion significantly reduced TCPOBOP-induced hepatomegaly and more remarkably hepatocyte proliferation. TCPOBOP-driven cell cycle activation was disrupted in YAP-KO mice due to delayed (and decreased) induction of cyclin D1 and higher expression of p21, resulting in decreased phosphorylation of retinoblastoma (Rb) protein. Further, induction of other cyclins, which are sequentially involved in progression through cell cycle (including cyclin E1, A2 and B1) and important mitotic regulators (such as aurora B kinase and polo-like kinase 1) was remarkably reduced in YAP KO mice. Microarray analysis revealed that 26% of TCPOBOP-responsive genes mainly related to proliferation, but not to drug metabolism, were altered by YAP deletion. YAP regulated these proliferation genes via alerting expression of cMyc and FOXM1, two critical transcriptional regulators of CAR-mediated hepatocyte proliferation. Our study revealed an important role of YAP signaling in CAR-driven hepatocyte proliferation; however, CAR-driven induction of drug metabolism genes was independent of YAP.

1927 The Role of Bromodomain and Extra-Terminal (BET) Proteins on APAP-Induced Hepatotoxicity

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Acetaminophen (APAP)-induced liver injury is a well-known model used to investigate genetic determinants of susceptibility to drug-induced liver injury (DILI) and acute liver failure. Antioxidant gene regulated by Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor, erythroid 2-like 2 (NRF2) pathway plays an important role in hepatoprotection against APAP-induced hepatotoxicity and oxidative stress. Recent studies have shown that bromodomain-containing protein 4 (BRD4), a bromodomain and extra-terminal motif (BET) protein, is a novel epigenetic regulator of KEAP1-NRF2 pathway. Inhibition of BRD4 using BET inhibitors was shown to upregulate antioxidant gene expression and protect against oxidative stress. Interestingly, the role of BET protein in DILI and acute liver failure is not well-studied. This study aims to understand these specific roles of BET protein using APAP-induced hepatotoxicity as a model. In this study, we used chemical inhibition and gene knock-down techniques to characterize the role of BET proteins against APAP-induced cytotoxicity using the immortalized mammalian cell lines HCO4 cells. Our results show that APAP treatment decreased BRD3 and 4 expression in HC-04 cells in a dose-dependent manner. Cytotoxicity analysis using LDH leakage assay showed that pretreatment of HC-04 cells with JQ1 before APAP administration significantly decreased APAP-induced cytotoxicity. Additionally, knockdown of BRD3 and 4 using siRNA transfections, protected against APAP-induced cytotoxicity. Moreover, gene expression analysis showed that inhibition of BET proteins upregulates the expression of genes involved in APAP detoxification pathways. In summary, our studies suggest that inhibition of BET proteins may confer protection against APAP-induced hepatotoxicity by upregulating genes involved in APAP detoxification pathways.

1928 Expression of Selective Autophagy Markers in Preneoplastic Liver Foci in High Fat Diet-Fed, Streptozotocin-Treated Rats

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Patients with nonalcoholic fatty liver disease (NAFLD) can be at risk for nonalcoholic steatohepatitis, which can lead to hepatocellular carcinoma. Autophagy protects against pathophysiological changes including steatosis and cancer. We determined the expression of selective autophagy markers in preneoplastic hepatic lesions and the effects of an autophagy inducer carbamazepine (CBZ) in a steatosis and diabetes mellitus-related early hepatocarcinogenesis model. Male F344 rats were fed a basal diet (BD) or high fat diet (HFD), and subjected to initiation and promotion steps with N-nitrosodiethylamine injection at week 0 and a partial hepatectomy at week 3. Several BD- or HFD-fed rats were received 30 mg/kg streptozotocin (STZ) at week 2. Some STZ-treated, HFD-fed rats were administered 200 mg/kg CBZ during week 7 and 8. STZ-treated rats developed diabetes mellitus, decreased body weight gain, and increased NAFLD score. STZ-treated, HFD-fed rats increased the area of glutathione S-transferase placental form-positive preneoplastic liver foci in the liver. CBZ tended to decrease STZ/HFD-increased preneoplastic foci and their Ki-67 labeling indices. An autophagosome marker LC3 and the LC3-binding protein p62 were heterogeneously expressed in the preneoplastic foci. CBZ might induce autophagy by significantly increased LC3 and decreased p62 in preneoplastic foci, in association with decreased expression of autophagy related genes, Atg5, Atg 6, Atg7, Lamp1, and Lamp 2. These results suggest that selective autophagy markers were specifically expressed in preneoplastic liver foci, and CBZ might decrease steatosis/diabetes-related early hepatocarcinogenesis by potentially inducing autophagy in STZ-treated, HFD-fed rats.

1929 Identification of Hepatocyte-Derived Exosome Biomarkers to Predict Idiosyncratic Drug-Induced Liver Injury

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We have previously demonstrated alterations in hepatocyte-derived exosomes (HDEs) prior to and in the absence of overt necrosis associated with idiosyncratic drug-induced liver injury (IDILI). HDEs contain miRNAs and proteins which may possess value in the form of sensitive and specific biomarkers for IDILI liability. The objective of this project was to identify HDE-based biomarkers of IDILI by profiling protein and miRNA changes in primary human hepatocytes exposed to subtoxic and toxic concentrations of IDILI compounds. Primary human hepatocytes were treated with increasing concentrations of 6 compounds known to elicit either idiosyncratic (tolcapone, isoniazid, or bosetan) or intrinsic (rotenone, acetaminophen and ethinyl estradiol) DILI via relevant cellular mechanisms. Subtoxic and toxic concentrations of each compound were selected from dose response curves and used for treatments in N=5 hepatocyte donors. After a 24 h exposure, HDEs were enriched from culture medium by ultracentrifugation, and changes in exosomal protein and miRNA were assessed using global profiling approaches. Significance was determined by an ANOVA model with linear contrasts between each compound concentration and DMSO control (p<0.05 and |fold change|> 1.5). Three proteins were significantly changed in response to both concentrations of all IDILI compounds: compliment factor 1 (decreased), inter-alpha-trypsin inhibitor heavy chain 1 (decreased), and vasolin containing protein (increased). All 3 proteins were readily quantifiable in HDEs by ELISA. Four miRNAs were significantly changed in response to the both concentrations of all IDILI compounds: miR-1244, miR-297, miR-548j-3p, and miR-576-3p (all decreased). Only miR-297 was detectable in reasonable sample quantities by qPCR in HDEs. In conclusion, we have identified 4 biomarkers of IDILI in HDEs which we are currently validating in studies with additional donors and eventually additional compounds. The findings of this study are novel as several of the candidate biomarkers have not been previously reported in HDE and/or to be altered in response to IDILI. Importantly, the HDE-based candidate biomarkers identified in this study may be utilized to refine existing *in vitro* assays for improving IDILI predictions.

1930 Vinyl Chloride Impacts Mitochondrial Dynamics and ER-Mitochondria Communication in Mice

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Vinyl chloride (VC), a common environmental pollutant, directly causing liver injury at high exposure levels. Importantly, we have shown that lower concentrations (i.e., < OSHA limits), which do not overtly damage the liver, enhance injury caused by high fat diet (HFD), at least in part, via mitochondrial dysfunction and ER stress. It has been shown that mitochondrial function and ER stress may be interconnected via ER-mitochondria interactions. These contact sites are sensitive to (patho)physiological conditions and maladaptive changes in this interaction have been associated with mitochondrial dysfunction. The purpose of the current study was to investigate the mechanistic impact of VC on ER-mitochondria interactions. C57Bl/6J mice were exposed to VC below the current OSHA standard (<1 ppm), or room air for 6 hrs/d, 5 d/wk for up to 12 wks. Mice were fed HFD, or low-fat control diet (LFD). Some mice received Alda-1 (20 mg/kg i.p., 3×/wk) for the last 3 weeks of diet/VC. Plasma and liver samples were collected for determination of injury and mitochondria were isolated for analysis of mitochondrial (dys)function. VC exposure exacerbated liver injury caused by HFD, reflected in increased transaminases, oxidative stress, and ER stress. VC exposure also dysregulated energy homeostasis and impaired mitochondrial function - even in the absence of HFD. Evidence for impaired mitochondrial function included dramatic structural changes to the mitochondria, impaired hepatic mitochondrial electron transport chain function and a decrease in maximum mitochondrial respiratory capacity, while fatty acid oxidation and mitochondrial DNA content were unaffected. VC also changed hepatic protein levels of several mitochondrial associated ER membrane (MAMs) proteins, which are involved in mitochondrial function, and quality control of ER-mitochondrial crosstalk, such as regulation of ER-mitochondrial calcium flux. Interestingly, mitochondrial dysfunction and changes to ER-mitochondria interactions, caused by VC and HFD, were diminished by ALDH2 allosteric activator, Alda-1. These results suggest a 'targeted' attack of mitochondrial function by VC, rather than overall nonspecific mitotoxicity. Taken together, VC dysregulates mitochondrial function/dynamics, ER stress and their interaction. These stress responses therefore play a causative role in VC-mediated liver toxicity and sensitization to other stressors (e.g. HFD). Importantly, these data raise concerns about potential overlap between diet and VC and emphasize that current safety restrictions may be insufficient to account for other factors that can influence hepatotoxicity in humans.

1931 Hepatotoxic Response in 2D and 3D Co-culture Models Differs from Hepatocyte-Alone Models

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It has become more widely accepted in both the regulatory and commercial fields that moving away from in-life rodent studies and toward testing species-relevant cells *in vitro* will provide alternative safety assessment strategies that are budget-conscious and alleviate ethical considerations. The liver has been a major focus of these efforts, yet there are currently no *in vitro* alternatives for hepatotoxicity testing accepted by regulators. An ideal *in vitro* liver model for hepatotoxicity testing would include hepatocytes and the non-parenchymal cells (NPCs) (ie. hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells), and would support hepatocyte viability, phenotypic maintenance, and metabolic competence for an extended time in culture to allow for repeated exposures and long-term dosing. The current studies have focused on developing organotypic two-dimensional 96-well plate-based and three-dimensional alginate bead-based culture systems that include primary rat hepatocytes and NPCs and can support hepatocyte viability *in vitro* out to eight days in 2D and 28 days in 3D. Using several canonical hepatotoxicants, we have compared 2D and 3D hepatocyte-alone (mono-) and hepatocyte and NPC (co-) culture systems to determine the robustness of these models. After treatment with acetaminophen (APAP; 5mM, 10mM, 20mM) for 3 consecutive days, APAP-induced cytotoxicity was significantly mitigated in the co-culture model, compared to the mono-culture model. In addition, APAP-induced CYP expression was lower in the co-culture model compared to mono-culture. Transcriptomic analysis of 2D mono- and co-culture models revealed that co-culture response to phenobarbital (10uM) is more representative (32% increase in similarity of the ontology enrichment) of *in vivo* responses (using OPEN TG_GATEs *in vivo* data, 300mg/kg, 24h). Similar to 2D models, after 10 days of repeat dosing with phenobarbital (25uM-400uM) relative cytotoxicity is diminished in 3D co-culture models compared to mono-culture. These studies have demonstrated that the organotypic model can recapitulate rodent *in vivo* liver phenotypes observed in response to canonical hepatotoxicants and suggest that the co-culture model could be useful for testing the effects of compounds *in vitro* as an early stage alternative to in-life studies.

1932 Co-administration of the Cannabidiol-Rich Cannabis Extract and Acetaminophen Leads to Severe Liver Injury in Mice

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The goal of this study was to investigate the potential for a cannabidiol-rich cannabis extract (CRCE) to interact with the most common over-the-counter drug and the major known cause of drug-induced liver injury – acetaminophen (APAP) – in aged female CD-1 and young C57BL6/J mice. Gavaging with 116 mg/kg of cannabidiol (CBD) [mouse equivalent dose (MED) of 10 mg/kg of CBD] in CRCE delivered with sesame oil for three consecutive days followed by i.p. acetaminophen (APAP) administration (400 mg/kg) on day 4 resulted in overt toxicity with 37.5% mortality in CD-1 mice. No mortality was observed in CD-1 mice treated with 290 mg/kg of CBD+APAP (MED of 25 mg/kg of CBD) or APAP alone. In C57BL/6J mice, 8% mortality was observed in 290 mg/kg dose. Following CRCE/APAP co-administration, microscopic examination revealed a sinusoidal obstruction syndrome-like liver injury in CD-1 mice and pan-necrosis in both CD-1 and C57BL6/J mice. The severity of both correlated with the degree of alterations in physiological and clinical biochemistry end points. Mechanistically, glutathione depletion and oxidative stress were observed in the APAP-only and co-administration groups, but co-administration resulted in much greater activation of c-Jun N-terminal kinase (JNK) in both mouse strains. These findings highlight the potential for CBD/drug interactions, and warrant further research needed to understand safety profile of CBD.

1933 Temporal Course of JNK Activation after an APAP Overdose

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Mitochondrial oxidant stress, activation of c-Jun N-terminal kinase (JNK) and its translocation to the mitochondria are recognized to be important early steps in acetaminophen (APAP)-induced hepatotoxicity. APAP-induced hepatocyte necrosis is initiated in cells around the central vein and radiates outward, with surviving cells at the border of the necrotic area initiating a regenerative response to ultimately re-populate areas of necrosis. While early JNK activation and its role in amplification of APAP induced mitochondrial damage is well characterized, its relevance in the later events, is not well understood. To examine activation, translocation and persistence of JNK on mitochondria after an APAP overdose, C57BL/6J mice were treated with 300mg/kg APAP followed by sacrifice at 2, 6, 12 or 24h later and liver injury assessed by measurement of plasma ALT levels and liver histology. Liver mitochondria and cytosol were separated by subcellular fractionation, followed by western blotting for total as well as activated phospho-JNK. Robust JNK activation in the cytosol and translocation to mitochondria was evident within 2h after APAP, which persists until 6h. However, by 12 and 24h, phospho-JNK was not detectable in either cytosol or mitochondria, which had only minimal amounts of inactive JNK protein. The spatiotemporal changes in activated phospho-JNK levels were then examined in liver sections and intense JNK activation was evident in centrilobular cells within 2h after APAP overdose, which persisted in cells surrounding the necrotic area by 6h. However, phospho-JNK expression had disappeared by 12 and 24h after APAP at which time we have earlier shown that mitochondrial biogenesis is initiated in cells bordering areas of necrosis. To explore mechanisms behind the transient nature of JNK activation, the temporal expression of mitogen-activated protein kinase phosphatase-1 (MKP-1), which is a negative regulator of JNK and has been implicated in APAP hepatotoxicity was examined. MKP1 protein levels were found to be elevated beginning at 6h and peaking at 12h after APAP, which paralleled the decrease in activated JNK indicating that dephosphorylation of JNK by MKP1 could be contributing to the transient JNK activation. In conclusion, these results suggest that the transient nature of JNK activation is probably relevant to progression of the necrotic area after APAP and delayed activation of MKP-1 helps in modulating this process.

1934 Gene Expression Regulation by mTOR Complexes I and II in the Mouse Liver

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The mechanistic target of rapamycin (mTOR) is a key serine/threonine protein kinase that functions in complexes mTORC1 and mTORC2. mTORC1, originally discovered due to its sensitivity towards the mTOR inhibitor rapamycin, responds to extracellular growth factor signaling, nutrient abundance, and variety of stress signals. Downstream effects of mTORC1 signaling consist in the regulation of the balance between anabolism and catabolism, including regulation of autophagy, mitochondrial metabolic function, protein synthesis, and ribosome biogenesis. mTORC2, initially discovered as a rapamycin-insensitive complex of mTOR, responds to insulin, growth factor signaling, and inflammatory signaling such as tumor necrosis factor-alpha, with its downstream effectors being Akt, a key serine/threonine kinase that functions in cell division, the NFkB pathway, and cytoskeletal reorganization and protein synthesis. Ability of mTOR pathway to integrate variety of stress (ROS, UPR, hypoxia) and nutrient signals (amino-acids, glucose, lipids, insulin) and convert them into downstream regulation of metabolism makes it central to the regulation of liver physiology. Little is known however about the role of mTOR signaling in liver response to toxic exposures. One knowledge gap that hiders this progress consists in non-complete understanding of unique and overlapping functions of the two mTOR complexes. In this study, we analyzed changes in gene expression in mouse models with liver-specific suppressed mTORC1 or mTORC2 complexes using RNA-seq approach. These models were generated by breading transgenic animals with Cre recombinase controlled by the Alb promotor and animals with floxed Raptor (component of mTORC1) or Rictor (component of mTORC2) respectively. Bioinformatic analysis of gene expression data counterintuitively demonstrated that functions positively regulated by mTOR complexes are activated by suppression of these respective complexes. That finding suggests presence of redundant mechanisms that likely overcompensate suppression of mTOR complexes. We have also found that in both KO models different pathways of lipid metabolism and protein biosynthesis and TNF-a signaling via NFkB were enriched. While mitotic cycle and xenobiotic metabolism were enriched in mTORC1 KO model only.

1935 Correlation of Physiochemical Properties of CC001 and Its Metabolites with Lysosome Accumulation in Rats

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Lysosomal sequestration is a well-known phenomenon of basic, lipophilic compounds. In an exploratory toxicity study, male Sprague Dawley rats (CRL:CD(SD)) dosed with CC001 at 50, 150, and 500 mg/kg/day, for 7 days had bile duct hyperplasia, increased macrophage vacuolation in liver and lymphoid tissue, and increased LAMP-2 staining in hepatocytes. Evaluation of tissues by electron microscopy showed evidence of phospholipidosis, as evidenced by concentric lamellar bodies, in bile duct epithelial cells but not in Kupffer cells or hepatocytes in the liver. These findings were absent in dogs and cynomolgus monkeys with studies of CC001 at equivalent or higher exposures. We initially hypothesized that a rodent-specific metabolite was responsible for the findings in the rat exploratory study, however, no apparent differences in parent:metabolite ratios or tissue specific concentrations of parent or metabolites was observed across species. We used *in vitro* cultures of the rat hepatoma cell line H4IIe as well as primary rat and dog hepatocytes to determine if CC001 or two of its metabolites, CC001a and CC001b, could disrupt lysosomes and induce vacuolation *in vitro*. Cells were incubated with CC001, CC001a, CC001b or the lysosomotropic compound chloroquine for up to 24 hours and disruption of lysosomes was measured by fluorescence microscopy of lysotracker red (LTR, Lysotracker Red DND-99) staining. We found that both the parent molecule, CC001, and the CC001b metabolite dose-dependently increased LTR labeling of rat H4IIe cells and rat hepatocytes. The extent of LTR increase in both H4IIe cells and hepatocytes corresponded to the physicochemical properties of the compounds: CC001a (cLogP = 4.31, pKa = 1.8, 8.7) < CC001 (cLogP = 4.5, pKa = 3.0, 4.7, 7.0) < CC001b (cLogP = 4.76, pKa = 9.2). Preliminary data suggests differential sensitivity of dog hepatocytes to CC001 and CC001b induced lysosomal disruption.

1936 Hepatocyte Exposure to Acetaminophen Induces DNA Damage, Epigenetic Changes, and Senescence

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Liver cells exposed to high levels of acetaminophen (APAP) accumulate by-products of metabolic pathways. Accumulation of reactive species in hepatocytes induces oxidative stress and alters genetic stability for hepatocytes. This leads to gene expression changes, as hepatocytes cope with their newly-imposed environ due to APAP exposure. In this study, gene expression data from non-malignant hepatocytes and a human liver cancer cell line (HepG2) exposed to between 2 and 10 mM APAP were interrogated for further insights. All microarray data were obtained from the Gene Expression Omnibus: non-malignant hepatocytes from *Homo sapiens* (GSE13430), *Rattus norvegicus* (GSE40336), and *Mus musculus* (GSE18614) as well as HepG2 cells (GSE53216). The Bioconductor *GEOquery* and *siggenes* packages were used in the R programming environment for processing data and for hypothesis testing, respectively. Furthermore, over-representation and Gene Set Enrichment analyses (GSEA) were performed. In both rats and humans, 5mM APAP over 24 hours increased the expressions of genes including those associated with *DNA Damage- /Telomere Stress-Induced Senescence*, and epigenetics-relevant processes such as *DNA methylation*, *SIRT1 negatively regulates rRNA expression*, and *PRC2 methylates histones and DNA*. Others include *Formation of the beta-catenin:TCF transactivating complex*, and *ERCC6 (CSB) and EHMT2 (G9a) positively regulate rRNA expression*. In mice, exposure to 2 mM of APAP over 24 hours, led to changes in expression of 214 genes, affecting 19 cellular pathways similar to those observed in rats and humans at 5 mM. Similar pathways were over-represented in up-regulated genes in HepG2 cells following 10 mM APAP exposure for 24 hours except that *p53-Dependent G1 DNA Damage Response* and *p53-Dependent G1/S DNA damage checkpoint* were also affected. GSEA results show a *DNA Repair* gene set is enriched in control hepatocytes, compared to 5 mM APAP-exposed ones, while *DNA Damage- /Telomere Stress-Induced Senescence* and *Oxidative Stress-Induced Senescence* gene sets are enriched in the 5 mM APAP-exposed ones compared to control. Furthermore, the gene set capturing *Methylation of Histones and DNA by PRC2* is enriched in 5mM APAP-exposed hepatocytes. Thus, hepatocyte APAP exposure for 24 hours likely increases DNA damage, while suppressing DNA repair, leading to epigenetic changes and senescence.

1937 The Involvement of Ryanodine Receptors in Halothane-Induced Liver Injury in Mice

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Drug-induced liver injury (DILI) is a major problem in drug development and clinical drug therapy. However, it remains difficult to predict DILI in humans due in part to the lack of suitable animal models and knowledge of susceptibility factors that predispose individuals to DILI. Halothane (HAL), an inhaled anesthetic, induces severe and idiosyncratic liver injury. It has been known that disordered hepatic calcium homeostasis is an early feature of HAL-induced liver injury in guinea pigs. Even though some underlying mechanisms in HAL-induced liver injury have been understood, there are no reports of involvement of ryanodine receptors (RYR) that mediates calcium release. To investigate the mechanism of HAL-induced liver injury, ryanodine (RYA, 50 ug/kg, i.p.), a RYR agonist, which was administered 1 h prior to HAL (15 mmol/kg, i.p.) with BALB/cCrSlc female mice significantly elevated plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, while resulted in severe hepatic inflammation and necrosis by histological analysis at 24 h compared with HAL-administered group. In contrast, administration of mice with dantrolene sodium (DAN, an inhibitor of RYR) showed a dose-dependent manner to suppress HAL-induced elevation of the plasma ALT and AST levels significantly, as well as alleviated the liver damage. Among the immune and inflammatory factors, the hepatic mRNA levels of TGF-β1, S100A8, macrophage inflammatory pryrotein-2, vascular cell adhesion molecule 1 were significantly increased in RYA and HAL co-administered group, but decreased in DAN and HAL co-administered group compared with HAL-administered group. In addition, hepatic mRNA expression levels of apoptosis markers including Bcl2-associated X, Bcl-2-interacting mediator and tribbles-related protein 3 were significantly decreased in RYA and HAL co-administered group compared with HAL-administered group. These results suggest that RYA has an enhanced effect and DAN has a protective effect on HAL-induced liver injury. In conclusion, we found a new insight into the effect of RYR which might be as a novel factor involved in the analysis of the mechanism on HAL-induced liver injury.

1938 Disturbed Microsomal TG Trafficking Contributes to Hepatic Fat Accumulation in Different Mouse Fatty Liver Models

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In liver endoplasmic reticulum (ER), newly synthesized triglyceride (TG) is transferred by microsomal triglyceride transfer protein (MTTP) to apolipoprotein B (apoB), which is matured in Golgi bodies to very low density lipoprotein (VLDL) and secreted into blood. If the synthesized TG does not bind to apoB to be secreted out of liver, the TG might be accumulated in liver forming fatty liver. In *ob/ob* mice, as expected, we observed an elevated TG contents in liver. Likewise, hepatic expression of MTTP and a lipid droplet-associated protein (Plin2) was increased dramatically. In contrast to liver TG and Plin2, liver apoB expression was decreased, but serum apoB and TG were not increased in *ob/ob* mice, suggesting that the decreased liver apoB in *ob/ob mice* is not due to a elevated apoB secretion. The observation that liver MTTP was increased but apoB was decreased in *ob/ob* mice with fatty liver leads us to further test the role of microsomal TG trafficking in another fatty liver model-binge alcohol-induced fatty liver. Mice were gavaged 5 g/kg of ethanol. Six hours later, like what we observed in *ob/ob* mice, liver TG and Plin2 were increased, liver apoB was also decreased. However, unlike *ob/ob* mice, binge alcohol-treated mice did not alter liver MTTP expression. Interestingly, in binge alcohol model serum apoB and TG were decreased, which was not observed in *ob/ob* mice. These results indicate that decreased liver apoB and increased Plin2 are essential for fatty liver in both *ob/ob* model and binge alcohol model. Previously we reported that peroxisome proliferator-activated receptor α (PPARα) deficiency promotes alcoholic fatty liver in *cyp2a5*$^{-/-}$ mice i.e. fatty liver was more severe in PPARα and CYP2A5 double knockout (P-A-) mice than in *cyp2a5*$^{-/-}$ mice still expressing PPARα (P+A-). Here we found that similar to what we observed in chronic alcohol model, binge alcohol-induced liver TG accumulation was higher in P-A- mice than in P+A- mice. Consistently, hepatic Plin2 expression was higher in P-A- mice than in P+A- mice. However, unlike P+A- mice, P-A- mice did not show a decreased liver apoB after a binge alcohol gavage. Surprisingly, liver MTTP was not detectable in control and alcohol treated P-A- mice, suggesting that PPARα regulates liver MTTP expression, and downregulated MTTP may play a pivotal role in the enhanced fatty liver in P-A- mice. To summarize, disturbance in microsomal TG trafficking (either MTTP or apoB) contribute to the development of fatty liver.

1939 Predictive and Mechanistic *In Vitro* Assays for Identifying DILI Potential

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Drug-induced liver injury (DILI) is one of the highest causes of drug attrition. *In vitro* cytotoxicity assays and several mechanistic assays with multiple endpoints were proposed as potential solutions to improve predictability of DILI in early phase of drug development. In this study, we assessed the utility of four *in vitro* cell-based assays, HepG2- and 3D human liver microtissue (hLiMT)-based ATP-driven cytotoxicity assay, HepaRG-based GSH consumption assay and liver humanized mice-derived human hepatocytes-based bile acid (BA) transport inhibition assay either alone or in combination for DILI risk assessment. We then compared the assay results with clinical information on DILI and previously published physicochemical property-based DILI risk classification (Biopharmaceutical Drug Disposition and Classification System:BDDCS;Benet LZ et al., AAPSJ, 2011) which classify extensively permeable, low aqueous solubility drugs as high risk. Eighty licensed DILI-causing and DILI-negative drugs were evaluated by these 4 assays. Previously published results were used for hLiMT-based cytotoxicity assay (Proctor WR et al., Arch Toxicol. 2017). The results showed that *in vitro* cell-based assays in combination were able to predict DILI with sensitivity range of 53.7-61.1%; specificity range of 76.9-84.0%; accuracy range of 63.3-66.3%. All the false positive drugs in *in vitro* assays except one drug in BA transport inhibition assay showed the therapeutic C_{max} values lower than 1 μM and sufficiently high margins. Notably, *in vitro* assays and BDDCS class 2 (high risk) detected different DILI-causing drugs as positive with overwrap, suggesting that potential DILI risk caused by extensive permeability and low solubility could not be fully captured by *in vitro* assays tested. Our data indicated that *in vitro* cell-based assays and/or physicochemical property-based assessment could contribute to drug candidate prioritization in early phase of drug development, and correction of assay results with predicted human exposure might be effective to improve predictive power when available.

1940 Expanded Primary Human Liver Sinusoidal Endothelial Cells as a Tool for Complex Hepatotoxicity Studies

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Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells lining the walls of hepatic sinusoids. Their key roles include liver regeneration, the transfer of substrates between blood and liver parenchyma, rapid internalization of blood-borne macromolecules as well as immune tolerance. Despite their substantial contribution to liver homeostasis, LSECs are often overlooked during hepatotoxicity assays due to insufficient cell yields after isolation and a restricted proliferation capacity *in vitro*. To address these issues, we expanded primary LSECs derived from 3 donors by lentiviral transduction with proliferation inducing genes. Transduced LSECs performed 28-45 population doublings in a donor-dependent manner until senescence occurred. Generated upcyte LSECs expressed typical endothelial cell markers (CD31, von Willebrand factor) and showed marked binding of UEA-1 (Ulex Europaeus Agglutinin I). In addition, we found expression of several LSECassociated receptors including MMR (mannose receptor), LYVE-1 (lymphatic vessel endothelial hyaluronan receptor 1) and FCGR2B (inhibitory receptor for the Fc region of immunoglobulin gamma). Expanded LSECs further revealed marked uptake of macromolecule ligands (ovalbumin, acetylated low density lipoprotein) and were capable of tube formation when cultured in Matrigel. Since LSECs are involved in drug-induced liver injury, we challenged the cells with several hepatotoxic model compounds. We further developed a co-culture medium which allowed us to analyze the interplay with hepatocytes. Interestingly, upcyte LSECs were more susceptible to e.g. acetaminophen and imipramine-induced toxicity when compared to upcyte hepatocytes, indicating that these cells constitute a useful tool to complement hepatotoxicity evaluation. Taken together, our data suggest that upcyte LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions. Future applications may include e.g. *in vitro* uptake assays of ADCs (antibody drug conjugates) or modulation of T cell activation in response to specific antigens.

1941 Immune-Mediated Hepatotoxicity: Novel *In Vitro* Model to Advance Drug Safety Testing

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Current *in vitro* liver models could greatly benefit from the incorporation of human liver macrophages - Kupffer cells (hKCs) due to their crucial role in the response of the liver to injury caused by drugs and other xenobiotics. Inclusion of hKCs have been limited due to challenges associated with the cell source: tissue availability, cost, donor variability and inability to expand the cells *in vitro*. In order to circumvent these challenges, we recapitulated *in vivo* ontogeny of hKCs to generate human iPSC - derived KCs (iKCs) and incorporated them in a human inflammatory *in vitro* liver model (HINVIL) with human iPSC-derived hepatocytes (iHeps). HINVIL was characterized by assessing performance of iHeps and iKCs in the model using marker expression (qPCR/FACs), cytokine production and phagocytosis assays. HINVIL was exposed to several inflammation-associated drugs including Acetaminophen, Trovafloxacin and Chlorpromazine. iKCs in HINVIL expressed macrophage markers CD32, CD68, CD11, CD14, CD163 at 0.3-5 folds of primary hKCs and KC-specific markers CLEC-4F, ID1 and ID3. iKCs produced cytokines IL-6 and TNFα and phagocytosed at levels similar to primary hKCs. iHeps in HINVIL expressed key hepatic markers (albumin and α1-antitrypsin) and key metabolizing cytochrome P450 (CYP) enzymes at 2.4-10.8 folds higher levels compared to iHeps mono-culture. Importantly, HINVIL could recapitulate *in vivo* like drug sensitivity by being able to detect hepatotoxicity at close to physiological concentrations of the tested drugs. In contrast, mono-culture of hepatocytes without the presence of iKCs did not show any improvement in the sensitivity of detecting these drugs. Furthermore, HINVIL could capture changes in cytokine levels (particularly decrease in IL-6 and increase in TNFα) produced by KCs in response to these drugs and consequent effects on important hepatic CYPs such as CYP3A4. Altogether, HINVIL comprises of renewable, mature and functional cells and represents a novel system for detecting immune-mediated hepatotoxicity.

1942 Oxidation of Fatty Acids Induced by PPAR-α Is Counteracted by PPAR-γ Activation in Mice with Hepatic Steatosis

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Peroxisome proliferator activated receptors (PPARs) have been shown to modulate many important pathways related to lipid metabolism in the liver. We have previously demonstrated that oral administration of PFOA in mice activates PPAR-α. We have also shown that the antibacterial compound triclosan (TCS) activates both PPAR-α and PPAR-γ. We examined these two compounds for their effect in our liver dietary steatosis model in mice. A HFD was administered *ad libitum* to male C57BL/6J mice for 32 weeks. After 16 weeks, when hepatic steatosis is established, the mice were treated with 1 mg/kg/day PFOA or 100 mg/kg/day TCS via diet for 2, 8, and 16 weeks. Histopathological examination revealed a reduction in the size and number of hepatic lipid vesicles of PFOA-treated mice in the HFD groups at all time points, but no change was observed in TCS-treated mice. This observation is supported by a significant reduction in hepatic triglycerides across all time points in HFD groups treated with PFOA, but not with TCS. Acyl-CoA oxidase activity (ACO) and *Cyp4a10* expression, as a measure of PPAR-α activity, were significantly increased in both PFOA and TCS-treated groups at all timepoints. However, ACO activity in HFD-fed mice was approximately twice as high when treated with PFOA compared to TCS despite having similar activity in mice fed the control diet. Total hepatic RNA was isolated for RNA sequencing and subsequent pathway analysis. Ingenuity Pathway Analysis revealed that in HFD groups both PFOA and TCS enriched many of the same pathways related to lipid metabolism including "oxidation of lipid" and "oxidation of fatty acid." The PPAR-γ pathway was significantly enriched as an upstream regulator of HFD-fed mice in the TCS group, but not the PFOA group. Across all time points mRNA expression of *Pparγ* was significantly increased in all TCS groups but not PFOA groups. Pathways related to fatty acid transport including "hydrolysis of lipid" and "activation of fatty acid" were significantly enriched when treated with PFOA but not TCS in the HFD groups. This data indicates that fatty acid oxidation induced by PPAR-α activation is blunted by PPAR-γ activity due to dysregulation of fatty acid transport mechanisms. Although PPAR-α activation primes liver machinery for fatty acid metabolism, simultaneous activation of PPAR-γ prevents the release of free fatty acids from the adipose tissue and are not available for transport to the mitochondria for oxidation.

1943 Microphysiological System for Studying Fatty Liver Disease and Its Impact on Drug-Induced Liver Injury

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Non-Alcoholic Fatty Liver Disease (NAFLD) is a growing concern worldwide and is set to become the most predominant cause of chronic liver disease. Additionally, there is a growing awareness of the potential risk factors for drug induced liver injury (DILI) due to the underlying metabolic condition. DILI in NAFLD patients can be exacerbated in two distinct ways; some drugs seem to aggravate pre-existing NAFLD, whereas more frequently others can induce acute hepatitis. However, the mechanisms by which these processes occur are poorly understood and adverse drug responses due to fatty liver are becoming more common with the ever-increasing obesity epidemic. Therefore, we have developed an advanced *in vitro* model to explore the relationships and mechanisms that link DILI and NAFLD. Using a microphysiological system (MPS), we have developed a fully human perfused *in vitro* NAFLD model, utilising primary human hepatocytes cultured in 3D to mimic the liver microarchitecture. Cells are cultured with high concentrations of free fatty acids for up to four weeks to induce intracellular triglyceride (fat) accumulation and mimic hepatic steatosis. Fat loaded cells can then be dosed with compounds of interest, potentially for extended periods of time to assess the effects of acute or chronic dosing. Fat loading of hepatocytes did not induce cell death or apoptosis, but impacted significantly on their metabolic capacity, reducing CYP3A4 (>2 fold) and CYP2C9 activity, whilst increasing CYP2E1 (>2 fold) and CYP1A2 activity (1.5-fold). We therefore explored if fat loaded hepatocytes were more sensitive to DILI with a range of compounds identified clinically to have exacerbated DILI in the presence of NAFLD. We observed both ticlopidine and acetaminophen to cause increased LDH release, decreased cell viability and decreased albumin production in fat loaded cells, compared to healthy/lean controls when compounds are dosed at or around IC:50 concentrations. We demonstrate the MPS *in vitro* NAFLD model is ideally suited to exploring the molecular mechanisms that underlie DILI and its association with hepatic steatosis. It will be a highly useful tool for analysing the toxicity profiles of novel compounds and how they may behave in diverse patient subsets.

1944 Gender Differences in Diet-Induced Nonalcoholic Steatohepatitis (NASH) in Cyp2b-Null Mice

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Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease; however, it is the progression to nonalcoholic steatohepatitis (NASH) that is associated with most adverse outcomes. CYP2B metabolizes multiple xeno- and endobiotics, and male Cyp2b-null mice are diet-induced obese (DIO) with increases in NAFLD but little hepatic inflammation. However, this DIO-study was only performed for 10-weeks and recent research indicates that C57Bl/6 mice are recalcitrant to fibrosis and NASH development for up to 22-weeks. Therefore, to properly assess the role of Cyp2b in fatty liver disease progression from NAFLD to NASH, we treated wildtype (WT) and Cyp2b-null mice with a normal diet (ND) or choline-deficient, L-amino acid-defined high fat diet (CDAHFD) for 8 weeks and determined metabolic and molecular changes. CDAHFD-fed WT female mice gained more weight and had greater liver (11%) and white adipose tissue (44%) mass than their Cyp2b-null counterparts, while males experienced diet-induced weight loss regardless of genotype. In both genders, hierarchical clustering of RNA-seq data demonstrates several genes involved in lipid metabolism, fibrosis, and inflammation responded differently in CDAHFD-fed Cyp2b-null mice compared to WT mice. Serum biomarkers of liver injury increased in both CDAHFD-fed female and male mice; however, CDAHFD-fed Cyp2b-null females exhibited significantly lower serum alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase concentrations compared to WT mice, indicating the loss of Cyp2b protected mice from liver injury in females. In contrast, males showed few differences in serum parameters related to genotype. Oil Red O staining and direct triglycerides measurements confirmed that CDAHFD-fed Cyp2b-null females were protected from fatty liver disease. CDAHFD-fed Cyp2b-null mice showed equivocal changes in fibrosis with some markers suggesting less inflammation probably due to glucocorticoid-mediated repression of immune responses. In contrast, CDAHFD-fed Cyp2b-null males had higher triglyceride levels. Results indicate that female Cyp2b-null mice are less susceptible to NASH than WT mice, while male Cyp2b-null mice are more susceptible to NAFLD with no changes in NASH development. Overall, this study indicates a minor role for Cyp2b in fatty liver disease progression that differs based on gender, with males being more susceptible to chemical inhibition of Cyp2b than females, who may be protected.

1945 Use of 2D and 3D *In Vitro* Models to De-Risk Potential for Liver Toxicity by BACEi BU

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JNJ-54861911 (JNJ-1911; Atabecestat) is a BACE inhibitor that caused Drug Induced Liver Injury (DILI) in the clinic. Resulting increases in liver enzymes (ALT) halted the program from further development. In order to avoid related issues with the backup compounds, we set off to understand the mechanism of JNJ-1911 induced DILI. This included generating the hypothesis, testing the hypothesis and designing model(s) to screen backup compounds. We hypothesized that oxidative stress induced by parent and/or reactive metabolites is a key contributor toward JNJ-1911 mediated DILI. To test this hypothesis, three separate *in vitro* methods were implemented: 1) Emulate's liver-on-chip, 2) Liver spheroids, and 3) Bio IVT HepatoPac. All models were treated with JNJ-1911 with or without glutathione inhibitor, Buthionine Sulfoximine (BSO). In addition, liver-on-chip and HepatoPac cultures were treated with competitor BACEi compounds with reported ALT increases (Lily LY2886721; JNJ-334) or no DILI (Merck Verubecestat; JNJ-548). Using APAP and aspirin as positive and negative controls respectively, we show that depletion of glutathione results in reduction of ATP and hepatic cell viability when treated with Atabacestat and LY2886721, but not Verubecestat, further suggesting a role of oxidative stress/reactive metabolite in initiation of DILI.

1946 Molecular Markers of Non-Alcoholic Fatty Liver Disease Variability in a Collaborative Cross Mouse Population

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Nonalcoholic fatty liver disease (NAFLD) is a fast-rising and the most prevalent form of chronic liver diseases in the United States. Interindividual variability and sexual dimorphisms in the development of NAFLD are still poorly understood. In the present study, 24 strains of male and female Collaborative Cross (CC) mice, a state-of-the-art mouse population model, were fed a high-fat and high-sucrose (HF/HS) diet or an ingredient-matched low-fat and reduced sucrose control diet for 12 weeks to investigate interindividual- and sex-specific variations in the development of NAFLD. The severity of liver steatosis in HF/HS diet-fed mice varied between sexes and individual strains, with a greater magnitude of hepatic steatosis found in male mice. This was evidenced by facts that (*i*) 68% of male strains showed a significant increase in fat liver accumulation as compared to 58% of female strains, and (*ii*) the inter-strain magnitude of steatosis was 66% in male mice, ranging from 3% to 69%, versus 27% in female mice, ranging from 4% to 31%. These changes were accompanied by an elevation of serum NAFLD diagnostic markers, including increases in serum total cholesterol, low-density lipoproteins, high-density lipoproteins, phospholipids, and glucose levels. The development of NAFLD was accompanied by over-expression of *Pparg, Mogat1, Cd36, Acaab1, Fabp2,* and *Gdf15,* and critical fatty acid uptake and *de novo* lipogenesis genes in male and female mice, among which the expression of *Pparg, Mogat1,* and *Cd36* was positively correlated with liver triglycerides in male mice, and *Mogat1* and *Cd36* expression was positively correlated with liver triglycerides in female mice. There was also an association between susceptibility to NAFLD and histone H4K16 deacetylation. In summary, our results indicate the value of the genetically diverse CC mice in combination with HF/HS diet-induced alterations to study the pathogenesis of NAFLD. This combination demonstrates the strength of our approach in the identification of molecular determinants of NAFLD pathogenesis and progression at the population level.

1947 Effects of Common E-cigarette Compounds in a Mouse Hepatocyte Model and Glutathione as a Potential Modulator of Susceptibility

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In 2017/2018, 2.9% of adults reported having used an e-cigarette at some point. More concerning is that 20.8% and 4.9% of high school and middle school students, respectively, also reported using e-cigarettes at least once. However, comparatively little research has been done on the potential hazards and effects of e-cigarettes. Four specific chemicals have been commonly associated with vaping: the pyrolysis products formaldehyde and acetaldehyde; and the flavoring agents cinnamaldehyde and diacetyl. In addition to assessing cytotoxicity caused by these agents, we are investigating whether the antioxidant glutathione (GSH) modulates susceptibility. *In vitro* experiments were carried using two immortalized mouse liver cell lines (wild-type cells, and GSH deficient Gclm null/β-galactosidase knock-in cells). Cell viability at multiple doses of each compound was measured using the alamarBlue assay after 24 hr. DDAOG, a β-galactosidase substrate, was used as a proxy for Gclm promoter activity, also at 24 hr. Formaldehyde, cinnamaldehyde, and diacetyl all resulted in an initial increase in viability at low doses followed by a subsequent dose-dependent decrease in viability by 2-way ANOVA. However, after correcting for multiple comparisons, only formaldehyde and cinnamaldehyde treatments caused decreases in cell viability. Interestingly, cinnamaldehyde treatment caused a dramatic decrease in viability in Gclm null cells at 250 μM, followed by a resurgence in survival until the 750 μM dose. Acetaldehyde showed very little effect on cell viability. β-galactosidase activity in GSH-deficient cells suggested an increase in Gclm promoter activity at lower, relatively non-toxic doses for all 4 compounds, providing preliminary evidence that they cause oxidative stress. Investigation of oxidative damage, GSH redox and transcriptional responses are ongoing. *Supported by NIH grants R01CA239253 and P30ES007033.*

1948 ratHepatoPearls: Encapsulated Rat Hepatocyte Spheroids for Better *In Vitro In Vivo* Correlation (IVIVC)

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Through the development of HepatoPearls®, Cyprio validated an *in vitro* 3D liver model based on spheroids of primary human hepatocytes encapsulated in alginate pearls. HepatoPearls® show physiologically relevant functions such as CYP450 enzymes and urea and albumin production with high cell viability over 6 weeks. They offer a long-term, highly consistent solution for many applications, in particular in preclinical ADMET studies. HepatoPearls® were therefore developed with human cells to obtain relevant *in vitro* characterizations in line with human trials. However, it can be of primary interest for drug manufacturers to compare *in vitro* 3D animal results with the historical database generated from small animal trials. In addition, 2D cultured rat hepatocytes are known to lose functions and viability even earlier than 2D human cells. Thus, we investigated here the development of rat HepatoPearls® as a 3D liver model to help correlating *in vitro* and *in vivo* results obtained on rodents. Rat primary hepatocytes were encapsulated with Cyprio's BioPearls® technology. Different initial cell densities and culture medium were compared to ensure spheroid formation, cell viability (Live/Dead) and liver functions (CYP P450 activity and bile canaliculus staining) during 4 weeks after encapsulation. With an increase in cell density compared to human HepatoPearls®, homogeneous encapsulation was successfully achieved and spheroid formation was validated. The spheroid stability over time showed important variations depending on the used medium. For optimal composition, rat HepatoPearls® were formed earlier than human ones (day 4 instead of 7) and were stable for 4 weeks with similar viability rate. Metabolic functionality was also confirmed through induction of CYP3A4 activity (up to 13-fold at day 4) and bile canaliculus observations. Interestingly, CYP3A4 basal activity tended to stabilize over the four weeks of study and the rat HepatoPearls® were inducible during all this period with 8.8, 5.5 and 5.7-fold of induction respectively at days 10, 17 & 24 In summary, we report here the first production of encapsulated ratHepatoPearls® with high cell viability and metabolic activity. The ability of providing reliable rat *in vitro* liver model with more than 1-week metabolic stability is a crucial improvement for *IVIVC*. We will investigate further the application of this new *in vitro* rat 3D liver model in hepatotoxicity studies.

1949 Transcriptomic Profiling of Human 3D Liver Spheroids Treated with Three Discontinued BACE Inhibitor Drugs

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Janssen recently ended clinical development of the BACE inhibitor Atabecestat since elevations in liver enzymes were detected in patients taking the drug. Dating back several years, Eli Lilly halted clinical development of its BACE inhibitor LY2886721 because of liver abnormalities. Also Merck stopped Verubecestat due to lack of desired pharmacology but without any indications of human liver toxicity. To assess the underlying mechanisms of the clinical drug-induced liver injury (DILI), a mechanistic *in vitro* study was set-up to explore the transcriptomic profiles induced by the 3 BACE inhibitors. In this proof-of-concept approach, 3D human liver spheroids were treated with different concentrations of each BACE inhibitor based on the clinical Cmax and on the cytotoxicity profiles obtained in a range finder study. Differentially expressed genes and pathways were compared after a 1- and 12-day exposure period. After treatment with Atabecestat at 3x and 10x Cmax, a significant deregulation of gene expression and pathway perturbations were observed, while the 2 other BACE inhibitors showed limited to no effect. Differences between the transcriptomic profiles generated at day 1 and 12, illustrated the dynamic nature of the human liver cell responses. The obtained transcriptomic data set was then analyzed using the Predictive Toxicogenomics Space (PTGS) model, a compacting and data fusion-derived tool to predict adverse outcomes and risk of human DILI from *in vitro* hepatocyte experiments. Based on the resulting PTGS-derived DILI scores after the 12-day treatments, the analysis could discriminate between the clinical DILI potential of the 3 BACE inhibitors. It can be concluded that the integrated use of a 3D human *in vitro* liver model, transcriptomic profiling, pharmacokinetic-based exposure calculation and PTGS modeling, can predict clinical DILI risk. The proposed test strategy needs further validation but is considered a promising approach to prioritize new drug candidates early in Discovery and/or select safe back-up compounds.

1950 A Systematic US FDA-Approved Drug High Content Imaging Screen to Uncover Drug Liabilities for Cellular Stress Response Pathway Activation

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Adverse Drug Reactions (ADRs) account for 5-10% of all hospital admissions. The most common drugs that cause ADRs are cardiovascular drugs, NSAIDs and central nervous system agents. Over the past decades, research has been focusing on individual drugs to understand mechanisms responsible of specific ADRs. In this project, an unprecedented large screen is performed to link the most frequent ADRs to the drug-like chemical space. This can lead to a better prediction of drug safety based on the chemical structure of a compound. Two libraries of in total 1965 drugs including all FDA approved drugs as well as 378 kinase inhibitors were screened with a panel of HepG2 BAC-GFP reporters to quantify cellular stress response activation involving DNA-damage (p21), ER stress (CHOP), oxidative stress (SRXN1) or inflammatory signaling (ICAM1); onset of cell death was followed by staining the samples with annexin V and propidium Iodide. In order to include as many compounds as possible to study the mechanism of toxicity, cells were exposed to 50 µM for all of the compounds. More than 600 compounds did activate at least one of the cellular stress response reporters. We observed an equal distribution pattern of reporter activation between the two libraries for p21-GFP (10% of the compounds from both libraries), CHOP-GFP (20%) and SRXN1-GFP (5%). Inhibition of the inflammatory pathway was predominantly caused by KIs, whereas enhancement of the response was mostly observed after treatment with FDA approved drugs. To validate the effect of the hits on a therapeutic dose level, a secondary screen was performed with dosages in a range of 0.3 - 50 µM. Here, 350 compounds were confirmed to activate the stress response pathway in a dose-dependent way. For some groups of structurally similar compounds, identical biological responses are activated. Our current data allows the classification of different FDA approved drugs and KIs for their dose-response dependent activation of individual stress response pathways. To gain more mechanistic knowledge, the pathway dynamics landscape has been extended by measuring protein-GFP expression for different modulators of the pathways. Furthermore, QSAR methods are being applied to define structural alerts for particular pathway activation.

1951 Hepatic Steatosis Shifts Phase I Metabolism and Increases Susceptibility to Toxicants *In Vitro*

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Hepatic steatosis (fatty liver disease) is a pathological condition that can alter xenobiotic metabolism, and thereby, alter susceptibility to environmental toxicants. Here we used a metabolically competent human liver-derived cell line - HepaRG - to model steatosis for *in vitro* toxicity assessment. Cells were exposed to vendor-provided completed-media with added 1% BSA-complexed oleate and palmitate fatty acids to induce a steatotic state. An optimum free fatty acid (FFA) ratio of 1:2 oleate to palmitate fatty acid, total FFA concentration of 1 mM, and a 7-day FFA incubation time were identified experimentally and chosen to achieve measurable lipid accumulation with minimal toxicity. The hepatotoxic piscicide rotenone was selected to assess chemical toxicity in our steatotic model. Cell viability was measured 24h after exposure using Cell Titer Glow, an intracellular ATP, assay. The IC50's for rotenone were altered with lipid loading, shifting a naïve IC50 from 0.64 µM to 0.46 µM in a steatotic model. The altered cell viability becomes increasingly significant, as seen using a two-tailed students t-test where $p<0.05$, with increasing concentrations of rotenone. There was a reduction in expression of several cytochrome P450 (CYP) genes in the HepaRG cells in a steatotic state when measured by qPCR. For example, CYP3A4, the most active P450 enzyme in rotenone metabolism, expression was reduced. P450 activity is a major factor in limiting rotenone toxicity as rotenone metabolites are less active than the parent compound. These results suggest that our *in vitro* HepaRG steatosis model can be a useful tool for evaluating *in vivo* hepatic steatosis as a risk factor in chemical toxicity. Future addition of high content analysis of oxidative stress and mitochondrial dysfunction in the model may enhance its predictive capability for human hepatotoxicity susceptibility screening. *This abstract does not necessarily reflect the policy of the US EPA.*

1952 *In Vivo* Effects of Endogenous AhR Ligands within the Intestine-Liver Axis

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Anthropogenic aryl hydrocarbon receptor (AhR) ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) cause the dose-dependent progression of steatosis to steatohepatitis with fibrosis in mice. Several endogenous ligands of the AhR have also been identified, however their *in vivo* hepatic effects remain unexplored. In these studies, male C57BL/6 mice were orally gavaged with the endogenous tryptophan metabolites - kynurenine (KYN; 0, 10, or 50 mg/kg dissolved in acidified water) or 6-formylindolo[3,2-b]carbazole (FICZ; 0, 1, or 4 mg/kg dissolved in sesame oil). Liver and intestinal epithelium samples were collected 2, 4, and 24 hours (h) following treatment. KYN did not alter body weight, relative liver weight (RLW), or hepatic histopathology. Although the expression of classic AhR battery genes was unchanged in the liver, *Cyp1a1* and *Cyp1a2* were induced in the jejunal epithelium 4.8- and 4.0-fold, respectively, at 50 mg/kg KYN. In contrast, FICZ induced hepatic expression of *Cyp1a1* (4180.7-fold), *Cyp1a2* (9.7-fold), and *Cyp1b1* (19.9-fold) at ≥ 1 mg/kg. At 24h, *Cyp1a1* and *Cyp1b1* expression returned to near basal levels, while *Cyp1a2* induction persisted throughout the study. Similarly, RLW was increased ~20% at 2 and 4h, but not at 24h. Dose-dependent increases in serum ALT levels at 2h suggest FICZ induced liver damage, while hepatic induction of the *Cd36* fatty acid transporter may promote lipid accumulation. Overall, the early hepatic effects elicited by 1 mg/kg FICZ are qualitatively and quantitatively similar to those elicited by TCDD, but lack the persistence reported with poorly metabolizable ligands. These studies demonstrate that some labile endogenous, dietary, and/or microbial ligands elicit effects that may contribute to the AhR–mediated progression of steatosis to steatohepatitis with fibrosis. *Funded by the National Institutes of Health ES029541.*

1953 Advanced Glycation End Products Induce Lipid Accumulation through Hepatic Lipid Uptake and *De Novo* Lipogenesis Pathway in Hepatocyte

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Nonalcoholic fatty liver disease (NAFLD) is known as chronic liver disease and the most prevalent liver disease, worldwide. Metabolic syndrome, type 2 diabetes, and insulin resistance are main causes of NAFLD, which is the first step of the development of cirrhosis. Five different advanced glycation end-products (AGE1 to AGE5) were prepared by a non-enzymatic reaction between the reducing sugars, such as glucose, glyceraldehyde, glycolaldehyde, methylglyoxal, and glyoxal, respectively and the protein, bovine serum albumin in this study. The formation of AGEs was widely detected in food and was generally known that induced chronic renal disease, type 2 diabetes and its complications. Although there is high relevance between causes of NAFLD and AGEs, the study that impact of AGEs on fat accumulation is insufficient. This study investigated that the AGEs augmented lipid accumulation that resulted from *de novo* lipogenesis and hepatic lipid uptake pathway in HepG2 cells. The mixture of oleic acid and palmitic acid was used as positive control. Increased Nile red binding, indicator marker of lipid accumulation, was observed after treated AGEs. The mRNA and protein expression of lipid synthesis markers such as PPARγ, FASN, and FAPB were up-regulated in AGEs treated cells. Furthermore, the AGEs regulated the mRNA and protein expression lipolysis marker such as CPT-1, and CD36. These findings suggest that AGEs may induce NAFLD and chronic liver injury through an imbalance between lipid acquisition and disposal.

1954 Using Transcriptomics Combined with 3D Microtissues to Predict DILI

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Drug-induced liver injury (DILI) is a central problem in drug development programs that typically results in late stage clinical trial failures, precautionary warnings and the post-marketing withdrawal of drugs. A variety of *in vitro* approaches have been used in an effort to improve prediction of DILI. Transcriptomics has been shown to play an important role in the understanding of cellular pathways and their alterations in stress situations, whilst also outlining the transcriptional alterations that occur as a result of chemical exposure. Moreover, toxicity assays utilising the combined approaches of

High-Content Analysis (HCA) and phenotypically relevant three-dimensional (3D) microtissue cultures allows deeper interrogation of the most important features contributing to liver toxicity (mitochondrial function, GSH, oxidative stress, cytotoxicity). Mechanistic understanding of DILI can be gained by combining the most predictive and physiologically relevant *in vitro* models with analysis through high-throughput RNA sequencing to deliver more comprehensive toxicity profiles. The screening of twenty-eight drugs with and without clinical DILI was assessed across eight doses in a long-term (14 days) HepaRG 3D model utilising high-throughput (HTS) RNA-seq and multiplexed high content screening (HCS) with mitochondrial membrane potential, GSH, oxidative stress and cellular ATP endpoints. The transcription profiles obtained allowed grouping of DILI positive and negative compounds into functional clusters by PCA (principal component analysis) and t-SNE analysis. A Large impact of gene expression, in a dose-dependent manner, was observed for DILI compounds and the degree of gene expression (DGE) was DILI rank dependent. Multi-parametric HCS in 3D microtissues allowed the identification of DILI compounds with 81 % of sensitivity, 86% specificity and 82 % of accuracy. In summary, predictive toxicogenomics where more comprehensive profiles can be drawn and combined with established mutiplexed *in vitro* assays, using human 3D hepatotoxic models, deliver early and late stage event identification in the hepatotoxic process. Such combination provides insights in the mechanisms implicated in drug toxicity, with heightened predictive understanding of DILI.

PS 1955 Novel Insight into the Dysmetabolic Effects of Olanzapine

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Olanzapine (OLZ), a second-generation antipsychotic, is effective in the treatment of schizophrenia and other mood disorders. However, these beneficial effects of OLZ are limited by its associated side effects, such as weight gain, metabolic syndrome and increased risk of cardiovascular disease. These effects are compounded by the fact that obesity is exceedingly prevalent in the psychiatric population. Most studies thus far have focused on the central effects of OLZ on dysmetabolism, but peripheral effects on key metabolic organs (e.g., liver) may also have important implications. Previous work by this group suggested that OLZ exposure *in vivo* directly alters hepatic metabolism, which contributed to organism-level dysmetabolism. The goal of the current study was to build on these findings both *in vitro* and in an animal model of obesity-induced dysmetabolism. 8-week old female C57BL/6 mice were fed either a high-fat or low-fat control diet (HFD and LFD). Mice also received either OLZ (8 mg/kg/d) or vehicle by osmotic minipump for 4 weeks. AML12 cells were exposed to OLZ (25 μM) for up to 24 h. The impact of OLZ exposure on metabolism was tracked by metabolic flux analysis (Seahorse) and via metabolomics analysis of cell lysate. OLZ alone increased mouse body weight, without a commensurate increase in food consumption. OLZ also caused hepatic steatosis and injury. Combining OLZ and HFD caused further dysregulation of glucose and lipid metabolism. Liver damage from concurrent HFD and olanzapine was worse than liver damage from HFD or OLZ alone. OLZ exposure directly inhibited oxidative phosphorylation in AML-12 cells, which was paralleled by a commensurate increase in lactate levels. As observed *in vivo*, OLZ also increased glutamine levels in AML-12 cells, which was coupled with an increase in mTOR phosphorylation. All of these effects *in vitro* reflect changes observed after *in vivo* exposure, in the presence and absence of an obesogenic diet. Taken together, these data suggest that OLZ directly impacts hepatic metabolism and exacerbates dysmetabolism. Importantly, directly targeting peripheral effects of OLZ may spare beneficial effects of OLZ in the central nervous system.

PS 1956 Hepatocytes Proliferation Studies with Primary Rat Hepatocytes and Primary Human Hepatocytes

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Many chemicals, classified as peroxisome proliferators, are known to increase the number of peroxisomes, which is accompanied by DNA replication and liver growth in rat and mouse hepatocytes. This in consequence can induce hepatocellular tumors. Hence, chemicals which induce the proliferation of peroxisomes have formed a unique class of chemical carcinogens, despite being initially classified as non-genotoxic. It has been shown that certain species differences are apparent in response to peroxisome proliferations. Rats and mice appear to be the most sensitive while humans appear to be relatively insensitive or non-responsive (Lai Dy, 2004). These species differences may be reproduced *in vitro* using primary culture hepatocytes isolated from a variety of species including humans. In response to high interest in hepatocyte testing Eurofins BioPharma Product Testing Munich developed hepatocytes proliferation assay using both primary human and primary rat hepatocyte cells. Primary rat hepatocytes were obtained using the method based on two-step collagenase perfusion developed by Berry and Friend (1969) and modified by Seglen (1976). Primary human hepatocytes were obtained from Sigma Aldrich. The quality of hepatocytes-culture was evaluated; preparations with viabilities below 90% were rejected. Cells were maintained in culture for 4 to 5 days and supplemented with hEGF for positive control. Proliferation of hepatocytes was determined using EdU incorporation into genomic DNA of proliferating cells. EdU-incorporation was than detected using click reaction with azide, conjugated with fluorescent dye: 6-FAM Azide. For the primary rat hepatocytes cells, we noticed an increase of 44.02 ± 14.27% in EdU positive cells in the dose group incubated with 50 ng/mL hEGF (96h treatment) in comparison to the negative control 0.45 ± 0.48%. Human primary hepatocytes showed an increase of 45.47 ± 18.24% in EdU positive cells in the dose group incubated with hEGF (72h treatment) in comparison with negative control 7.67 ± 3.58%. This assay allows for quick and reliable evaluation of the effect of test materials on hepatocytes proliferation. The hepatocyte proliferation assay is a universal method that can be applied for each identified target without extensive developmental work.

PS 1957 Gene Expression Analysis, Quantitative Proteomics, and Chronic Toxicity Studies in Primary Mouse Hepatocyte Spheroids Support the Development of an *In Vitro* Collaborative Cross Platform for the Evaluation of Genetic Susceptibility Factors Associated with DILI

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We are developing an *in vitro* platform using primary mouse hepatocytes isolated from the genetically diverse lines of the Collaborative Cross and cultured as 3D spheroids to enable the rapid and cost-effective investigation of gene-by-treatment interactions associated with drug-induced liver injury (DILI). The objective of this study was to compare the spheroid model to native liver and fresh hepatocytes using global gene expression profiling and quantitative targeted proteomics (QTAP) and to demonstrate time and concentration-dependent toxicity responses to DILI drugs. Microarray profiling of the entire transcriptome and QTAP assessing 10 important drug metabolizing enzyme and transporter (DMET) proteins were performed on liver, hepatocytes, and corresponding hepatocyte spheroids cultured for 1, 7, and 14 days post spheroid formation from N=3 male and female C57BL/6 mice. Differences among models were determined by ANOVA with linear contrasts (FDR $p<0.05$ and $|FC|>1.5$). In total, 1073 of 12826 genes (8.4%) measured in spheroids were differentially expressed compared to liver and hepatocytes. However, only 52 genes (0.4%) were differentially expressed in spheroids over time and no changes were observed in mRNAs encoding the 10 important DMET proteins. Correspondingly, the levels of 9 of 10 important DMET proteins measured by QTAP were decreased in spheroids compared to hepatocytes and liver. However, only 1 of the 10 DMET proteins (Cyp2e1) was decreased in spheroids over time. Time and concentration-dependent decreases in ATP and albumin were observed in response to acetaminophen, fialuridine, AMG-009, and tolvaptan with albumin responses to chronic exposures observed for 3 of 4 drugs (all but acetaminophen) at <1 μM concentrations. Collectively, these results demonstrate maintenance of mRNA and DMET protein levels from days 1-14 post spheroid formation and the ability to show responses to DILI drugs at clinically relevant concentrations in chronic toxicity studies using the spheroid model.

1958 Effect of Low- and High-Fat Diet on Lipidomic Blood Changes Induced after *In Vivo* Exposure of Male C57BL/6 Mice to Perflorooctane Sulfonic (PFOS) and Perflourohexane Sulfonic Acid (PFHxS)

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Millions of people are exposed to per- and polyfluoroalkyl substances (PFASs) exposure every day through diet and it is known that there is an association between exposure to these PFOS and hepatic steatosis. However, there is a gap in knowledge about the association between changes in blood lipids and exposure to PFASs, as well as lack of knowledge about the effects of low and high fat diet on both hepatotoxicity and blood lipids. We addressed these gaps by exposing C57BL/6 mice to perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) in low-fat diet (LFD (11% kcal from fat)) and high-fat high carbohydrate diet (HFD (58% kcal from fat)) for 29 weeks. Changes in the blood lipidome were analyzed using both an untargeted shotgun approach (electrospray ionization-mass spectrometry (ESI-MS)), and a targeted quantitative approach (HPLC-ESI-Orbitrap-MS/MS). Blood was isolated from C57BL/6 mice exposed to either PFOS or PFHxS (~0.3mg/kg/day) via low or high fat diet, and lipids were extracted from the blood using the Bligh-Dyer method. The initial untargeted ESI-MS approach demonstrated distinct clustering within the blood lipidome with the most dramatic shifts occurring between LFD and LFD with PFAS (L-PFOS and L-PFHxS) exposure and HFD and HFD with PFAS (H-PFOS and H-PFHxS) exposure. HPLC-ESI-MS/MS analysis revealed a total of 2,918 discriminatory ion features for lipids isolated from mice exposed to LFD, L-PFOS, L-PFHxS, HFD, H-PFOS, and H-PFHxS. Mice exposed to PFOS and PFHxS in the presence of a LFD had higher levels of phosphatidylcholine (PC), as compared to those only exposed to the LFD. 14:0-22:2 PC was enriched in the blood of mice exposed to L-PFHxS and L-PFOS as compared to LFD control mice. Surprisingly, plasmalogens were significantly enriched in mice exposed to H-PFHxS as compared to HFD control mice. These data demonstrate the novel finding that PFAS exposure alters the blood lipidome of mice after *in vivo* exposure, and suggests that the effect of PFAS's on the blood lipidome is diet-dependent. The serum lipidome findings indicate a correlation between dietary consumption and PFAS exposure, which may provide a basis for identification of PFAS related lipid predictors.

1959 Hepatocyte AhR Expression Mediates TCDD-Induced Hyaluronan Accumulation and Extracellular Matrix Degradation during Carbon Tetrachloride-Induced Liver Injury

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Liver fibrosis occur after relentless injury due to an imbalance between the synthesis and degradation of extracellular matrix (ECM) molecules, such as collagen and hyaluronan (HA). We previously found that aryl hydrocarbon receptor (AhR) activation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exacerbates liver injury, inflammation, and myofibroblast activation during experimental liver fibrosis induced by carbon tetrachloride (CCl_4). This effect of TCDD was abrogated in CCl_4-treated mice with AhR-deficient hepatocytes ($AhR^{\Delta Hep}$), but unexpectedly, the extent of liver fibrosis was not reduced in these mice. Our objective was to determine if increased HA content and/or reduced ECM degradation mediated the robust fibrosis observed in CCl_4/TCDD-treated $AhR^{\Delta Hep}$ mice. Male $AhR^{\Delta Hep}$ and $AhR^{fl/fl}$ mice were treated with 1.0 ml/kg CCl_4 every four days for five weeks, and TCDD (100 ug/kg) was administered during the final week of the experiment. Mice were euthanized at the end of the five-week period. A hyaluronan binding protein assay was used to localize hepatic HA, *in situ* zymography was performed to evaluate gelatinase activity, and RNA sequencing was used to interrogate gene expression. Results indicate that TCDD treatment increased HA content in the CCl_4-injured liver of $AhR^{fl/fl}$ mice but not $AhR^{\Delta Hep}$ mice. Increased HA correlated with increased gene expression for hyaluronan synthase-1 and the HA cell surface receptor, CD44, as well as decreased expression of hyaluronidase (*Hyal2*). *In situ* zymography showed that cotreatment with CCl_4 and TCDD increased gelatinase activity in $AhR^{fl/fl}$ mice. In contrast, there was no increase in gelatinase activity among any treatment groups in $AhR^{\Delta Hep}$ mice, and transcript levels for matrix metalloproteinases-8 and -9 were consistently reduced. These data suggest that hepatocyte AhR signaling mediates the TCDD-induced accumulation of HA in the fibrotic liver, as well as ECM degradation, the latter of which likely underlies the robust fibrosis observed in CCl_4/TCDD-treated $AhR^{\Delta Hep}$ mice. *Supported by P20GM103549 & P30GM118247.*

1960 Cryopreservation of Encapsulated 3D Micro-Livers, HepatoPearls, through Careful Optimization of Freezing and Thawing Processes

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Approval of new drug candidates is a long and costly process. It is unfortunate to fail during or after clinical trials due to undetected toxicity issues during preclinical studies. Indeed, performant cellular models providing reliable risk prediction and allowing for high-throughput *in vitro* screening are still lacking. Previously, Cyprio showed that HepatoPearl®, a 3D spheroid liver model encapsulated in alginate pearls, is a viable and metabolically functional human model for acute and chronic toxicity tests and DMPK studies. It ensures improved CYP450 enzymes activity over a longer lifespan (6 weeks) compared to 2D culture, as well as urea and albumin synthesis and bile efflux. To go further and provide a ready-to-use 3D cellular model for high-throughput screening, we cryopreserved HepatoPearls® to enable long-term in-lab storage. We compared 5 different protocols from faster to slower cooling rates and 2 thawing protocols. HepatoPearls® were cryopreserved accordingly immediately after production then kept in liquid $N_2$for 1 week before thawing, 1-month culture and analysis. We investigated alginate pearls integrity, Cyp3A4 enzymatic activity and induction, urea and albumin synthesis and cell viability in comparison with non-cryopreserved ones. With the fastest cooling rate, we observed 27.4% of altered pearls (respectively 3.6% destroyed and 23.8% damaged) post-thawing. However, with slower and more controlled cooling rates (multi-step processes), impacted pearls decreased to 6.8% (respectively 0.36% and 6.45%). Interestingly, the different thawing processes didn't affect the number of altered capsules, allowing us to validate a protocol similar to the thawing step of standard cryopreserved cells (37°C water bath). The cell functionality assessment showed that HepatoPearls® were still presenting equivalent levels of CYP3A4 expression over one month compared to non-cryopreserved samples. Moreover, spheroid integrity and cell viability remained unaffected. In summary, we validated the development of an efficient cryopreserved model of HepatoPearls® for high throughput *in vitro* hepatotoxicity tests. From a broader perspective, this is a major step towards an off-the-shelf human 3D liver model, physiologically relevant over 1 month and allowing for easy and adaptable study designs. Further developments to cryopreserve encapsulated liver cells from other origins (species and cell sources) are ongoing.

1961 Dose-Dependent Effects of 2,3,7,8-tetrachlorodibenzo-*p*-Dioxin (TCDD) on Acetyl-CoA Metabolism

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Acetyl-CoA is a two-carbon metabolite that plays a central role in carbohydrate, lipid, and protein metabolism. Its primary function is to deliver an acetyl group into the Krebs cycle leading to further oxidation and production of adenosine triphosphate (ATP). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a persistent environmental contaminant, has been shown to cause the development and progression of steatosis to steatohepatitis in mice resembling human non-alcoholic fatty liver disease (NAFLD). TCDD activates the aryl hydrocarbon receptor (AhR), a transcription factor responsible for regulating gene expression. TCDD causes central carbon metabolism reprogramming in mice, however the effects on acetyl-CoA metabolism have not been investigated. In this study, male C57BL/6 mice were orally gavaged every 4 days for 28 days with sesame oil vehicle control or 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 µg/kg TCDD. Hepatic acetyl-CoA levels and gene expression were examined using untargeted liquid chromatography tandem mass spectrometry and RNA-Seq analysis, respectively. At 3, 10, and 30 µg/kg TCDD, hepatic acetyl-CoA levels decreased 1.9-, 28.5-, and 6.4-fold, respectively. TCDD also repressed gene expression associated with β-oxidation, fatty acid biosynthesis, ketone body metabolism, and coenzyme-A metabolism. Decreased protein levels of ATP citrate lyase (ACLY) and short-chain acyl-CoA synthetase 2 (ACSS2) were confirmed, suggesting sources (e.g. citrate and acetate) and utilization (e.g. fatty acid biosynthesis) of acetyl-CoA were repressed. Depletion of acetyl-CoA is consistent with reduced protein acetylation and AMPK activation, indicating a hepatic energy deficiency which may compromise cell proliferation and antioxidant defenses. *Funded by the Superfund Research Program P42ES04911 and the National Institutes of Health ES029541.*

1962 Effects of 10 New Generation PFAS-Containing Aqueous Film Forming Foams (AFFF) and Single PFAS Compounds on Human Liver Cell Viability and Function

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals characterized by their carbon-fluorine bonds, and resistance to heat, water, and oil. PFAS have been used in hundreds of industrial applications and consumer products; a use they are well known for is in aqueous film forming foams (AFFF) to extinguish hydrocarbon fuel-based fires. Due to high stability and bioaccumulation of some PFAS in the AFFF, they have been measured in bodies of water and in human blood worldwide. The purpose of this study was to evaluate the cytotoxicity of 10 AFFFs, thought to be in current use in 2018, in a human hepatocyte/cholangiocyte cell line (HepaRG). Numerous studies have demonstrated that the liver is a particularly sensitive tissue to the adverse effects of PFAS. Metabolically-competent HepaRG liver cells were exposed to the AFFF by blinded allocation for 24 hours at a volume-per-volume concentration range of 0.0008% to 0.8% in media. CellTiter-Glo luminescence assay detected substantial declines in cellular viability at concentrations between 0.1% and 0.4%, and total cell death between concentrations of 0.5% and 0.8% for several AFFF. Several of the individual components that have been determined to be present in each of the AFFF, such as 6:2 fluorotelomer sulfonate (6:2 FTS) and non-ionic surfactants, such as sodium lauryl sulfate (SLS), were identified and evaluated for cytotoxicity. 6:2 FTS had an active range of cytotoxicity at approximately 100 uM to 300 uM. Additionally, although SLS was tested within the relevant range found in AFFF, it did not explain the effect of AFFF. Furthermore, lipid accumulation assays and H2AXP DNA repair assays were utilized for the evaluation of how these AFFF and individual PFAS compounds affect functional processes in exposed, metabolically-competent HepaRG liver cells. The AFFF and single PFAS compounds with the highest EC_{50} and least biological toxicities will be prioritized for testing *in vivo*, in hopes of identifying compounds that may serve as alternatives to the legacy PFAS compounds that are currently used in AFFF solutions.

1963 Fgf15 Overexpression and PPARα Activation as a Combination Therapy for NASH in a Mouse Model

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Non-alcoholic fatty liver disease (NAFLD) is a progressive disease that is increasing worldwide along with the obesity epidemic. Approximately 25% of the US population have NAFLD with up to 30% of that NAFLD population developing non-alcoholic steatohepatitis (NASH). Currently, there is no FDA approved treatment for NASH. One emerging therapeutic target for NASH is fibroblast-growth factor 19 (FGF19, mouse ortholog FGF15). This hormone is expressed in the small intestine and regulates bile acid synthesis and glucose homeostasis. Additionally, activation of peroxisome proliferator-activated receptor-alpha (PPARα) increases fatty acid oxidation in hepatocytes and decreases triglycerides. Therefore, the overexpression of FGF15 in conjunction with a PPARα agonist, was hypothesized to further reduce the severity of NASH in a mouse model. To investigate, 8 week old wild-type (WT; C57BL/6J) and transgenic *Fgf15* (*Fgf15*Tg) mice were maintained on a control diet (CD) or high-fat diet (HFD) for 6 months. PPARα agonist WY-14643 (CAS #: 50892-23-4) (0.05% w:w) was administered for 6 weeks to HFD groups once NASH was induced at 4 months. HFD feeding increased body weight in both genotypes although *Fgf15*Tg mice were more resistant to weight gain. Glucose tolerance tests (GTTs) showed *Fgf15*Tg mice were more glucose tolerant, suggesting they are more insulin sensitive, while HFD decreased glucose tolerance in both genotypes. Serum levels of cholesterol and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were decreased in *Fgf15*Tg mice after HFD feeding, suggesting hepatic protection. Activation of PPARα promoted weight loss and decreased serum levels of cholesterol, triglycerides, ALT, and AST. In contrast, alkaline phosphatase (ALP) activity was increased after PPARα activation. Combination treatment resulted in further weight loss, reduced serum levels of cholesterol, triglycerides, ALT, and AST; however, combination treatment did not further improve serum biochemical markers compared to individual treatments. *Funding: R21ES029258, BX002741, T32ES007148.*

1964 The Environmental Contribution to Nonalcoholic Fatty Liver Disease Severity

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease. This cross-sectional NAFLD study seeks to determine: (*i*) if environmental exposures are associated with the severity of steatosis/fibrosis; and (*ii*) if steatosis/fibrosis share any common metabolic pathways. The precision medicine approach applies high resolution exposomics/metabolomics to well-characterized NALFD patients. Archived de-identified plasma samples/data from 150 consenting adult NAFLD patients were utilized. Imaging biomarkers of steatosis (controlled attenuation parameter, CAP) and (liver stiffness measurement, LSM) were previously determined by Fibroscan®. LC-MS^2 was performed using a High-Field Q-Exactive mass spectrometer with C18 (negative ionization) and HILIC (positive ionization) columns. apLCMS and xMSanalyzer were used for data extraction. Linear regression analysis was performed on log2 transformed and quantile normalized data using R. Statistical significance was set at FDR<0.2. Pathway enrichment analysis was performed using mummichog 2.0. Metabolic features were annotated using xMSannotator and an in-house validated library. 65% of subjects were women and 51% were diabetic. Mean age and BMI were 51.4±12.4 (SD) and 36.0±7.4 kg/m^2. Mean CAP and LSM were 325.8±53.5 dB/m and 12.1±11.3 kPa. 30,800 features were detected by LC-MS^2. Of these, 272 were significantly associated with CAP and 271 with LSM. CAP was positively associated with features putatively assigned to pesticides (bromuconazole and methomyl) and hexabromodibenzofuran. Bromuconazole levels were 5.2-fold higher in the highest *vs.* lowest exposure quintile. LSM was positively associated with nicotine and features putatively assigned to pesticides (metolcarb and nitromethylidenehydrazinylbenzoic acid), a prescription medication (olopatadine) and copper benzoate. Metabolic pathways significantly enriched with CAP were: bile acid, carnitine shuttle, porphyrin, aminosugars and amino acids. Pathways significantly enriched with LSM included: porphyrin, carnitine shuttle, bile acid, C21 steroid hormones and other amino acids. Specific environmental exposures were associated with more severe steatosis and fibrosis in NAFLD patients. Steatosis/fibrosis shared several metabolic pathways. More precision medicine data are required to better understand the environmental contribution to NAFLD.

1965 Combined High Content Imaging in Liver Microtissues and Mitochondrial Toxicity to Predict DILI

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Drug induced liver injury (DILI) is a leading cause of drug attrition in drug development. A considerable aetiology of DILI is mitochondrial toxicity leading to withdrawal of drugs such as troglitazone and cerivastatin (1). In order to avoid such liabilities, an improvement in preclinical cell based models are vital that can predict the mitochondrial toxicity in early stages of drug development. Such models will reduce the late stage attrition of drug candidate and ultimately lead to safer drugs in the market. The approach of 3D liver models using human isolated hepatocytes and human non-parenchymal cells provides a physiological microtissue with prolonged cytochrome P450 activity up to 3 weeks in culture. The model combined with biochemical assay (ATP) has been shown as a sensitive and specific strategy to predict DILI (2-3). Here, we have optimized the method of producing reproducible liver microtissue (hLiMTs) and combined this with multi-parametric confocal high content imaging (HCI) allowing the determination of mitochondrial dysfunction (mitochondrial mass, mitochondrial membrane potential, mitochondrial specific ROS and cellular ATP). This 3D HCI approach was also combined with a mitochondrial stress test to determine mitochondrial liability using oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Combining both of these approaches indicated that mitochondrial effects in hLiMTs was implicated in 42% of known DILI compounds from a reference set of 62 compounds. Furthermore by taking all the endpoints into account the overall sensitivity and specificity of this approach was 87% and 100% respectively. This study illustrates the importance of considering mitochondrial liabilities in DILI prediction and in combination with 3D human liver models as a useful approach to de-risk DILI in early drug discovery screens. 1. Dykens JA and Will Y (2007) the significance of mitochondrial toxicity testing in drug development. *Drug Discovery Today* 12: 777-785 2. Proctor *et al;* (2017) Utility of spherical human liver microtissues for prediction of clinical drug drug-induced liver injury. *Arch. Toxicol. 91*(8), 2849-2863 3. Vorrink *et al; (*2018) Prediction of drug-induced hepatotoxicity using long-termstable primary hepatic 3D spheroid cultures in chemically defined conditions. Toxicol. Sci. *163(2), 655-665.*

1966 An Imaging-Based RNA-Interference Screen Reveals Novel Key Regulators of the Drug-Induced Endoplasmic Reticulum Stress Response

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Mechanistic understanding of drug-induced liver injury (DILI) is currently still lacking and therefore hard to predict. Some of these drugs induce endoplasmic reticulum (ER) stress and activate the unfolded protein response. However during chronic ER stress, activation of the UPR will be insufficient and will activate apoptotic pathways mediated by CHOP leading to hepatotoxicity. To improve mechanistic understanding, we aimed to identify novel key regulators of CHOP using a siRNA-screening approach. First, the suitability of the HepG2 CHOP-GFP reporter for evaluating CHOP induction was determined using various ER stress inducers. Secondly, a concentration of 6 µM of tunicamycin was found to be most optimal to study CHOP induction perturbations by siRNA silencing. Using these conditions, we applied an imaging-based RNAi screen of the druggable genome targeting 3457 genes in HepG2 CHOP-GFP cells to identify novel regulators of the tunicamycin-induced ER stress response. CHOP-GFP expression was evaluated after 16 hours of tunicamycin exposure which was altered by 201 genes upon knockdown from which 74 could be confirmed. These potential regulators were further evaluated with other ER stress inducers and their role in induction of other UPR-related genes such as ATF4, XBP1 and BIP. Next, their role in DILI compound-induced ER stress was determined, where potential regulators could affect significantly the UPR activation during DILI compound exposure. As validation, CRISPR/Cas9-mediated knockout showed similar effects on CHOP induction dynamics during ER stress. To evaluate the relevance of 10 selected novel regulators for the human liver during ER stress, we evaluated the transcriptome in both HepG2 and primary human hepatocytes (PHHs) after knockdown and subsequent exposure for 16 hours of tunicamycin. Three potential regulators were confirmed in PHHs which showed upon knockdown perturbation of UPR activation after tunicamycin. Pathway analysis revealed their key role in multiple ER signaling pathways, namely translation, protein degradation, UPR and protein trafficking. Overall, our RNAi screen allowed the identification of novel regulators of the drug-induced ER stress response and will further shape our understanding and prediction of DILI liabilities. *Supported by EU-ToxRisk project (grant agreement No 681002) and IMI MIP-DILI project (grant agreement 115336).*

1967 Differential Hepatic Recovery after Sub-Lethal Microcystin-LR Exposure in Healthy versus Nonalcoholic Steatohepatitis Rats

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Nonalcoholic steatohepatitis (NASH) is a progressive liver disease, and can advance to cirrhosis or hepatocellular carcinoma with continual exposure to exogenous stressors like toxicants or poor diet. Microcystin-LR (MCLR) is a hepatotoxic cyanotoxin known to cause a NASH-like phenotype, and exacerbate preexisting diet-induced NASH. Previous data indicate the liver can recover after MCLR-induced hepatotoxicity in healthy rodents. We hypothesize that MCLR-induced hepatotoxicity in diet-induced NASH will not recover due to continued exogenous stress from the poor diet. Male Sprague-Dawley rats were divided into two groups and fed a control or a high fat/high cholesterol (HFHC) diet for eight weeks. The animals were then subdivided into two treatment groups receiving intraperitoneal injections of 0.9% saline (vehicle) or 30 µg/kg MCLR every other day for two weeks. Sets of animals from each diet/treatment subgroup were euthanized after the MCLR exposure period, and subsequently at two or four weeks of recovery. Diet regimens were maintained for the entirety of the study. MCLR toxicity altered fasting plasma biochemistries in both healthy and NASH groups, which recovered to basal levels after four weeks. At the end of the two-week MCLR exposure period, hepatic apoptosis was evident in both control and NASH groups, as indicated by ~56% decrease in expression of Bcl-2 and up to ~130% and ~184% increase in cleaved caspases 3 and caspase 9, respectively. Protein phosphatase (PP) bound MCLR was observed by western blot after MCLR exposure in both diet groups, but was not observed after two and four weeks of recovery. A low molecular weight form of PP2A (~25 kDa) was observed following MCLR toxicity in both diet groups that persisted through two and four weeks of recovery despite turnover of the PP2A bound MCLR. MCLR-induced liver fibrosis resolved after four weeks of recovery in the healthy group, but persisted in the NASH group. Following four weeks of recovery, the MCLR treated NASH subgroup had less liver steatosis and more liver inflammation compared to the vehicle treated NASH subgroup. These data suggest that despite recovery in plasma biochemistries and resolution of apoptosis, continued stress of a poor diet following MCLR exposure impairs hepatic recovery mechanisms, and resembles a burnt-out NASH phenotype. *Funding: 4R00ES024455.*

1968 Construction of Hepatic Vascular Model and Toxicity Assessment System That Can Predict DILI Compounds

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Drug-Induced-Liver-Injury (DILI) is one of the most significant reasons drug development or sales are discontinued. Due to the complicated mechanism of DILI, it still has been far difficult to predict the toxicity before or even after clinical study phase. Researches on predicting such toxicity by using *in vitro* hepatic tissue constructed from human-derived cells have been developed in many groups. But it is also difficult to mimic highly ordered structure like blood vessels, and no hepatic-toxicity assessment system hasn't reached satisfactory level. The purpose of this study is constructing of a hepatic vascular model, and toxicity assessment system by using the model. At first, we constructed a liver-like tissue model consisted from human hepatocytes from xenogeneic host livers (PXB cell), Sinusoidal Endothelial Cell (SEC), and hepatic stellate Cell (LX-2). In this case, we applied our unique 3D culture method. This method can be characterized by mixing cells with collagen in a specific form. By performing immunostaining on the model and observed the morphological features by CLSM, we confirmed the multi-layered structure of blood vessels and hepatic cells. We also evaluated the model in terms of general function of liver, albumin secretion. The models were able to keep albumin secretion at least within 15 days without drastic decreasion. Furthermore, we confirmed the possibility that the model can be used for toxicity assessment by applying typical DILI compound. The model can predict the toxicity of DILI compound as similar to traditional 2D culture. In summary, we constructed a liver-like model having multi-layered structure of blood vessel and hepatocytes. The model kept albumins secretion within 15 days. We showed that the model can function at least as much to 2D culture. In future plan, we will characterize the model by defining unique toxicity assessment ability.

1969 Image-Based Quantification of Bile Canalicular Alteration in HepaRG Cultures for Prediction of Drug-Induced Cholestasis

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Hepatic cholestasis involves the abnormal accumulation of bile acids in bile canaliculi (BC) and is implicated as a one of mechanism of drug-induced liver injury. While it has long been suggested that inhibition of bile salt export pumps (BSEP) underlies such abnormal accumulation, more recent studies have found that known drug-induced BSEP inhibition is not the only mechanism of cholestatic DILI. It has been demonstrated that Rho kinase/Myosin Light Chain kinase pathway plays a key role in the alterations. Objective: Our objective was to provide a reliable, quantitative assay that instead more broadly characterizes BC dynamics with the aim of evaluating cholestatic risk more accurately. Through this study, this assay was validated using compounds with known cholestatic risk. HepaRG cells were cultured, treated with or without chlorpromazine and fasudil, and labeled with CDFDA. Images were acquired by fluorescence microscopy and thresholded to enable BC object detection and area quantification. Using automated image analysis, the area of bile canaliculi in treated samples were measured. It was shown that fasudil and chlorpromazine resulted in strong BC dilation and constriction, respectively. Fasudil caused a 44% increase in mean areas measured in HepaRG cells, whereas chlorpromazine induced 41% decrease in mean areas of BC with respect to control cells. Through the combination of a HepaRG *in vitro* model for cholestasis and a quantitative imaging approach to evaluating BC dynamics, this new approach represents a promising screening approach for the prediction of cholestatic liabilities.

1970 Drug-Induced Impairment of Mitochondrial Fatty Acid Oxidation and Steatosis: Assessment of Causal Relationship with 45 Pharmaceuticals

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Drug-induced liver injury (DILI) represents a major issue for some treated patients and for pharmaceutical companies, being a frequent cause of drug withdrawal from the market. Steatosis (lipid accretion) is one of the diverse liver lesions that can be induced by drugs. While microvesicular steatosis is rare and fatal, macrovacuolar steatosis is relatively common and benign, although it can progress to steatohepatitis and cirrhosis. Both types of steatosis can be secondary to an impairment of fatty acid β-oxidation (mtFAO), although such impairment is stronger in microvesicular steatosis. Importantly, mtFAO can be impaired by direct inhibition of mtFAO enzymes or indirectly via an inhibition of the respiratory chain activity. Of note, an overall causal relationship between steatosis and mtFAO inhibition has not yet been established with a high number of pharmaceuticals. Hence, 32 steatogenic and 13 non-steatogenic drugs were tested for their ability to induce direct or indirect inhibition of mtFAO in mouse liver mitochondria (MiToxView® platform; Porceddu *et al.,* 2018). To this end, we assessed oxygen consumption driven by substrates of complex I (malate/glutamate), complex II (succinate) and mtFAO (palmitoyl-L-carnitine, octanoyl-L-carnitine, or palmitoyl-CoA + L-carnitine), allowing us to specify the mechanism of mtFAO impairment, if any. Our multiparametric assays in isolated liver mitochondria revealed that steatogenic drugs frequently induced mtFAO impairment. Indeed, 25 out of 32 steatogenic drugs (78%) inhibited mtFAO with an EC_{20} lower than 400 µM. For some drugs (e.g. methyldopa and dexamethasone), mtFAO impairment was not related to an inhibition of the respiratory chain, contrary to amiodarone and perhexiline. Inhibition of mtFAO was confirmed in differentiated HepaRG® cells with some steatogenic compounds (e.g. indomethacin and rifampicin). Finally, mtFAO inhibition was observed with only 5 of the 13 non-steatogenic drugs (38%). However, such inhibition was in general weak, excepted for glimepiride. Porceddu M, Buron N, Rustin P, Fromenty B, and Borgne-Sanchez A. *In vitro* assessment of mitochondrial toxicity to predict drug-induced liver injury. In book: Methods in Pharmacology and Toxicology - Drug-induced liver toxicity, 2018 Eds. M. Chen & Y. Will, Springer LLC, New York (NY, USA).

1971 Transcriptomic Profiling of the Inter-Individual Variability of Drug-Induced Cellular Stress Response Activation Using a Large Panel of Primary Human Hepatocytes

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Drug-induced liver injury (DILI) remains a major concern for the clinic and pharmaceutical companies. Given the diversity of DILI outcomes, it is crucial to improve its prediction at an early phase in drug development by integrating mechanistic understanding. Many hepatotoxicants are known to induce adaptive stress responses, a cellular mechanism to overcome stress. Since some patients are more prone to develop DILI, it is key to map the inter-individual variability in activation of these stress responses. Therefore, we profiled the transcriptome of a panel of 50 cryo-preserved primary human hepatocytes derived from different individuals exposed for 8 or 24 h to a broad concentration range of tunicamycin for unfolded protein response (UPR), diethyl maleate for oxidative stress response, cisplatin for DNA damage response and TNFα for NF-κB signaling. Transcriptomic profiles were related to LDH leakage as a measure for cytotoxicity. The variance in the concentration-dependent stress response activation among individuals could be captured, where the average of point-of-departures (PoD) for UPR-related genes resulted in a maximum difference of 866 fold. For each stress response, hepatocytes were classified based on a sensitivity score using maximum fold change and PoD for pathway-related genes. Correlation of the sensitivity scores and their background such as disease status was identified. To eliminate that the de-differentiation rate is not the driver of the found variability in stress response activation, the transcriptome in liver tissue, suspension and 24 hour plated for a selection of hepatocytes was profiled. Next, the three most sensitive or insensitive hepatocytes for UPR or oxidative stress response were exposed to various DILI compounds. Here, sensitive hepatocytes showed higher upregulation of pathway-related genes for most of the DILI compounds compared to insensitive hepatocytes. In conclusion, profiling of the inter-individual variance in drug-induced stress response activation will aid in the improved understanding of the variance in susceptibility towards DILI among patients. *Supported by EU-ToxRisk project (grant agreement No 681002) and IMI MIP-DILI project (grant agreement 115336).*

1972 Fibroblast Growth Factor 15 (FGF15) Suppresses NASH Development in Mice

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Non-Alcoholic Steatohepatitis (NASH) is a severe progression of Non-Alcoholic Fatty Liver Disease (NAFLD) in which liver steatosis, inflammation and hepatocyte damage occurs. NASH is a prevalent public health concern on the rise with reports of 12% of individuals being affected with this disease in the United States. There are currently no treatments. Farnesoid X receptor (FXR), a nuclear receptor that is critical in regulating glucose and lipid metabolism homeostasis, is activated by bile acids. Previous research has found that FXR protects the liver from NASH progression. An intestinal FXR downstream target gene is fibroblast growth factor (FGF) 15/19. The role of FGF15 in NASH development is unclear. This current research determines the effect of intestinal FGF15 deficiency on NASH development using the conditional knockout (CKO) of intestinal *Fgf15* in a high fat diet (HFD)-induced NASH model in mice. Male *Fgf15* CKO mice were fed either control chow or high fat diet, and sacrificed at 1, 3, and 6 months for assessment of NASH progression. Results suggest that FGF15 decreases bile acid synthesis, hepatic inflammation, and liver fibrosis. In conclusion, FGF15 serves a vital player in gut-liver cross talk, most likely protecting the liver from NASH-related excess bile acid injury. As the NASH global prevalence increases, therapeutic research for possible drug targets is of utmost importance. *Supported by ASPET SURF, NIHR25ES020721 and R01GM104037.*

1973 Acute TCDD Exposure Produces NAFLD-Like Pathology and Gene Expression in Mice with Chronic Liver Injury

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Non-alcoholic fatty liver disease (NAFLD) consists of a spectrum of diseases divided histologically into two subgroups: nonalcoholic fatty liver (NAFL), which refers to steatosis without inflammation, and non-alcoholic steatohepatitis (NASH), which includes steatosis and necroinflammation with or without fibrosis. Patients in both subgroups are at risk for developing progressive fibrosis, which can lead to cirrhosis and hepatocellular carcinoma. NAFLD development and progression are influenced by metabolic disarrangements, such as obesity, type 2 diabetes, metabolic syndrome and insulin resistance. Increasing evidence indicates that exposure to environmental toxicants may also be a significant risk factor for NAFLD and NASH. We have previously shown that activation of the aryl hydrocarbon receptor (AhR) by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exacerbates liver necrosis, inflammation, myofibroblast activation, and fibrogenesis in a mouse model of chronic liver injury elicited by carbon tetrachloride (CCl_4) administration. In the present study, we expanded these findings to test the hypothesis that acute TCDD exposure produces a NAFLD/NASH-like condition in mice with CCl_4-induced liver injury. Mice were treated with 1.0 ml/kg CCl_4 every four days for five weeks to elicit chronic liver injury, and TCDD (100 µg/kg) was administered during the final week of the experiment. Histological analysis revealed that TCDD treatment elicited hepatic steatosis, necroinflammation, and fibrosis in CCl_4-treated mice. This collection of pathological endpoints was not observed in mice treated with CCl_4 or TCDD alone, and it was not observed in CCl_4/TCDD treated mice with AhR-deficient hepatocytes. RNA-sequencing analysis revealed that co-treatment with CCl_4 and TCDD produced a NAFLD-like gene expression profile in the liver that was dependent on hepatocyte AhR signaling. For example, TCDD treatment increased the expression of genes that promote triglyceride accumulation, and transcriptionally inactivated genes related to glycolysis, gluconeogenesis, glycogen synthesis, as well as insulin signaling. Based on these findings, we conclude that exposure to TCDD may provide the "second hit" required to produce a NAFLD/NASH phenotype in a previously injured liver, and this appears to be mediated by AhR-mediated signaling in hepatocytes.

1974 Time to Treatment after Plating Impacts PFAS Induction of Gene Expression in Cryopreserved Human Hepatocytes

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Per- and polyfluoroalkyl substances are man-made compounds that are ubiquitous in the environment. These compounds originate from the use of aqueous film forming foams (AFFF), non-stick, water-resistant household and personal items. Perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA), and perfluorohexane sulfonate (PFHxS) have all been linked to liver damage. PFASs have limited passive permeability and are known substrates for membrane transporters. Organic Anion Transporting Polypeptides (OATPs) and Organic Anion Transporters (OATs) to enter cells, especially hepatocytes and renal proximal tubules. It has been described that OATP expression decreases in 2-D cultured human hepatocytes the longer they remain in culture. Thus, plating time, which can impact OATP expression, could be a significant variable in predicting the relative potency of PFASs, especially for emerging PFASs that are poorly understood. Thus, the goal of this study was to observe whether treating sooner after plating the cryopreserved hepatocytes would induce a more robust transcriptional response compared to the typical 24-hour time point. It was hypothesized that if OATP and OAT transporter expression is higher at an earlier time point after plating (4 hours), then it would result in more uptake of PFAS into the hepatocytes, greater PFAS effects compared to the traditional 24hour time point. Cryostax 5-donor pool of cryopreserved human hepatocytes (HH) were cultured following the manufacturer's protocols. 4-hours after plating, the hepatocytes were treated with PFOS, PFOA, and PFHxS at a series of concentrations (10 nM, 0.025 µM, 0.25 µM, 2.5 µM, 25 µM). 24-hours after plating, a second set of human hepatocytes were treated with the same compounds at the same concentrations. After the 48-hour treatments, the cells were lysed and the gene targets were observed using a QuantiGene 2.0 Bioplex assay, and analyzed on a Bio-Rad Bio-Plex 200 platform. Preliminary results from this study indicate that for many prototypical PFAS targets, (i.e. Cyp4a14), the earlier time to treatment after plating caused a robust upregulation of gene expression at lower concentrations, whereas other pathways (i.e. oxidative stress) were more responsive to treatment at 24 hours after plating. This information may suggest that PFAS potency in hepatocytes may be dictated by factors, such as uptake transporters or other receptor-mediated mechanisms that get modulated by time to treatment after plating, thus demonstrating the importance of time plating for PFAS screening with HH.

1975 Predictive Identification of Drug-Induced Damaged Site in Urinary Organs by Comprehensive Urine Examinations in Comparison with Histopathology in Rats

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Urine contains many kinds of cells derived from urinary organs and biological substances. Examination of urine is useful to investigate conditions of urinary organs in the non-invasive way. Male Sprague-Dawley rats were intraperitoneally injected once with cisplatin (CDDP), 2-bromoethylamine (BEA), and cyclophosphamide (CP) which are known to cause proximal tubule, renal papillary, and urinary bladder injuries, respectively, in order to reveal characteristic changes in urinalysis data when different areas in urinary organs are injured. Urinalyses (urine volume, specific gravity, urine color, urine sediment, test paper, and urine chemistry) were performed prior to dosing and 1 to 4 days after dosing, and histopathological examinations on the kidney, ureter, and urinary bladder sampled at necropsy at 4 days after dosing were conducted. CDDP induced increased number of epithelial cells and casts and high excretion levels of glucose, albumin and N-acetyl-β-d-glucosaminidase (NAG) from 2 days after dosing, and these changes were consistent with necrosis in proximal tubules noted in histopathology. BEA induced increased number of epithelial cells, casts, and red blood cells, and high excretion levels of albumin and NAG from the following day of dosing. These changes were consistent with histopathological findings such as necrosis in renal papilla and tubular cast. CP induced ulcer and hemorrhage in the transitional cells of the urinary bladder histopathologically, and increased number of epithelial cells, white and red blood cells in red colored urine and increased excretion level of album in urinalyses from the following day of dosing. There were no histopathological changes in the kidney, and urine glucose and NAG levels were not changed in urinalyses in CP treatment rats. These results suggest that comprehensive evaluation of urinalyses items can be useful for predictive identification of drug-induced damaged site through urinary organs in the non-invasive way.

1976 Development of Human iPSC Derived Cells for Nephrotoxicity Testing

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The prevalence of kidney disease is increasing at an alarming rate. This increase has several aspects, including lack of early detection, metabolic diseases and aging populations. However, it is likely that environmental pollution and pharmaceuticals also have a major role. While animal models provide some prediction value, they are not well suited to cellular mechanistic studies. Also, species differences often play a major role in pharmaco/toxico kinetics and dynamics. Furthermore, humans are genetically diverse, with increasingly identified polymorphisms and gene copy number variations in xenobiotic handling genes (i.e. metabolism and transport). In the kidney, proximal tubular epithelial cells are constitutive transporters that transport substances from the lumen and thus play an essential role in the whole-body homeostasis. Many xenobiotics are also transported here and can reach the cytosolic concentrations not seen in other tissues which may cause injury leading to acute and chronic renal disease. In this study we explore the possibility of differentiating induced pluripotent stem cells (iPSC) into renal proximal tubular like (PTL) phenotypes and their utilization in high throughput screening of drugs and chemicals by testing their sensitivity to the known toxicants. iPSCs were treated with different small molecules (CHIR, TTNBP) and specific growth factors over two weeks transitioning through the intermediate mesoderm, renal vesicles and finally to cells resembling transporting epithelial cells. Temporal development of the tissues was characterized by assessing the expression of pluripotency markers, renal developmental markers and maturation markers via immunofluorescence, RNA sequencing and western blot analysis. The final PTL cells exhibit a polarized phenotype, barrier formation with the expression of tight junction proteins such as occludin and ZO3 and the proximal tubule specific marker, megalin. Cells also showed typical proximal tubular hormonal response such as sensitivity to parathyroid hormone and lack of sensitivity to vasopressin. These cells have also been successfully used for testing pharmaceutical activity and chemical-induced toxicity in transcriptomic and high content imaging assays. In conclusion, the protocols developed here represent a promising tool to explore individual genetic susceptibilities to potential nephrotoxins. *The project was funded by a Marie Sklodowska-Curie Action - Innovative Training Network, entitled in3, under grant no. 721975.*

1977 Dissecting Drug-Induced Toxic Effects on Cell Viability and Metabolic Activity in Proximal Tubule Cells

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Acute kidney injury (AKI) is a major disease burden, affecting twenty percent of hospitalized adults worldwide. Mortality rates are over fifty percent in severe cases and less severe manifestations are associated with chronic kidney disease. Twenty-five percent of AKI cases are drug-induced, emphasizing the importance of renal toxicity assessment. The 'gold standard' MTT assay is widely used as a measure of cell viability, but depends on cellular metabolic activity. Mitochondrial dysfunction is expected to play a major role in nephrotoxicity, thus MTT may not be the most optimal assay to assess cytotoxicity of nephrotoxic drugs. We aimed to compare MTT with a fluorescent microscopy approach to measure cell viability. Mature conditionally immortalized proximal tubule epithelial cells (ciPTEC) were exposed to nephrotoxic compounds, including cisplatin, tenofovir, sanguinarine, NSC-95397 at a 0.001-1000 µM range. After 24 hours, cell viability was assessed by MTT or nuclear staining, which was microscopically investigated using a mixture of hoechst, YO-PRO-1 and propidium iodide to detect all, apoptotic and necrotic cells, respectively. Differences were compared with one-way ANOVA and Dunnett's *post hoc*. MTT-based cell viability was reduced by 99.1 ± 0.5% (mean ± SEM, $p<0.0001$) after cisplatin exposure, whereas the microscopic approach only detected a 32 ± 4% ($p<0.0001$) decrease in viability, defined as increased number of apoptotic and necrotic cells. Similarly, tenofovir decreased viability with 68 ± 6% (MTT, $p<0.0001$) and 58 ± 9% (nuclear staining, $p<0.0001$). Sanguinarine and NSC-95397 showed residual viability of 1.4 ± 0.1% ($p<0.0001$) and 2.2 ± 0.2% ($p<0.0001$) using MTT, respectively. However, microscopically both compounds demonstrated a residual viability of 20 ± 10% ($p<0.0001$) and 18 ± 8% ($p=0.0012$), respectively. Currently, time-dependency of enhanced viability reduction by MTT compared to nuclear staining is investigated. This study shows that, compared to our fluorescent microscopy approach, the 'gold standard' MTT assay, does not solely assess cellular toxicity but rather provides a composite readout of cell death and decreased metabolic rate. A nuclear staining approach is thus preferable when assessing cytotoxicity of metabolically active compounds.

1978 Elevated Glucose Concentration Decreases the Expression of Lysosomal Proteins in Human Proximal Tubular Cells

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Hyperglycemia is one of the major health concern in many parts of the world. One of the serious complications of high glucose levels is diabetic nephropathy (DN) which is the leading cause of end stage renal disease, characterized initially by the appearance of microalbuminuria, followed by progression to overt proteinuria and an overall decline in renal function. In order to determine the molecular changes involved in the development of diabetic nephropathy, human proximal tubular cells were cultured in the presence of 7.5, 11 and 16 mM glucose for three passages, and microarray analysis was performed to determine the differential gene expression in the HPT cells. The preliminary data obtained showed a significant downregulation in expression of mTOR as well as some of its associated genes. In addition, there was also a decrease in expression of genes associated with the lysosome. The results of the microarray analysis was confirmed using RT-qPCR, Western blot analysis and confocal microscopy. The results validated the microarray analysis, which showed decrease in the mRNA as well as protein expression of the genes CCLN7, NPC2, LIPA, RRAGD, SQSTM1, mTOR, LAMP1, EIF4EBP1, RPTOR and RICTOR as the concentration of glucose increased. Co-localization of lysosomal marker, LAMP1 and mTOR gene showed lower expression of mTOR as the glucose concentration increased, suggesting decrease in mTOR activity. Although the mechanism by which glucose affects the expression of genes associated with the lysosome is not well known, our results suggest that high levels of glucose may lead to decrease in mTOR activity causing the cells to enter an anabolic state resulting in the downregulation of lysosomal genes.

1979 Transcriptomic Characterization of Renal Pathomechanisms in the ZSF1 Rat Model of Diabetic Nephropathy and Comparison to Toxin-Induced Chronic Kidney Disease

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Incidence of chronic kidney injury (CKI) is growing, but early diagnostics and treatment remain insufficient. Many causes of CKI have been identified, examples being nephrotoxins like aristolochic acid (AA) and metabolic diseases. Transcriptomic analyses in the context of CKI should improve our understanding of insult-specific (nephrotoxin vs. disease-associated) or common mechanisms. We therefore compare the renal gene expression in four rat models of CKI: (1) ZSF1 obese rats as a genetically induced model of diabetic nephropathy (DN), (2) L-NAME treated Renin-2 transgenic rats as a model of hypertension, (3) Wistar rats treated with nephrotoxic serum as a model for glomerulonephritis (Pavkovic et al 2014)., and (4) Big Blue rats treated with AA (Su et al 2011). All animals were compared to respective controls. ZSF1 gene expression profiles were furthermore compared to human DN (Woroniecka et al 2011) to assess its translational value. Renal damage was assessed by histopathology and proteinuria. RNA was subjected to Microarray (Affymetrix) hybridization followed by initial analysis using Genedata software. Upstream predictions were derived using Ingenuity pathway Analysis (IPA, Qiagen). In addition, co-expression signatures were determined with the Weighted Gene Co-expression Network Analysis (WGCNA) generated with the TXG-MAPr App, provided in the frame of IMI2 projects eTRANSAFE and TransQST. Kidney injury was confirmed by increased proteinuria and histological observations. On the transcriptome level, damage is mostly associated with similar deregulations across the different CKI models when pathways like fibrosis, inflammation, ROS responses and renal function are analyzed. This is supported by IPA upstream predictions. On the other hand, differences between the CKI models can be observed for other functions, like decreased oxidative phosphorylation specifically in ZSF1 rats. This is supported by WGCNA, which also predicts differences in gene co-expression networks. Comparison to human DN strengthens the translational value of the ZSF1 model. Our results highlight disease-specific and common pathomechanisms in rat models of CKI and the translational value of those models for mechanistic studies and biomarker research.

1980 Sulforaphane Prevents Type 2 Diabetes-Induced Nephropathy via AMPK-Mediated Activation of Lipid Metabolic Pathways and NRF2 Anti-Oxidative Function

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Sulforaphane (SFN) prevents diabetic nephropathy (DN) in type 2 diabetes (T2D) by up-regulating nuclear factor (erythroid-derived 2)-like 2 (NRF2). AMP-activated protein kinase (AMPK) can attenuate the pathogenesis of DN by improving renal lipotoxicity, which is also found associated with the activation of NRF2-mediated antioxidants. Therefore, we investigated whether AMPKα2, the central subunit of AMPK in energy metabolism, is required for SFN protection against DN in T2D, and whether potential crosstalk occurs between AMPKα2 and NRF2 in this context. AMPKα2 knockout (Ampkα2-/-) mice and wild type (WT) (C57BL/6J) mice were fed a high-fat diet (HFD) to induce insulin resistance, followed by streptozotocin (STZ) injection to induce hyperglycemia (T2D model mice). Both T2D and control mice were treated with SFN or vehicle for three months. At the end of the three-month treatment period, five mice from each group were sacrificed (three-month (3M) time point). The remaining mice were only fed either a HFD or normal diet (ND) for three more months and the study was terminated at six months (six-month (6M) time point). Renal function was assessed by measurement of 24h urine albumin before sacrifice at 3M and 6M. SFN significantly attenuated T2D-induced renal dysfunction and pathology accompanied with biochemical changes including proteinuria, renal hypertrophy, renal oxidative damage, inflammation, and fibrosis in WT mice, but not in Ampkα2-/- mice. Additionally SFN also prevented T2D-induced renal lipotoxicity via AMPK-mediated activation of lipid metabolic pathways and the NRF2 function in WT diabetic mice. Similarly, these protective effects of SFN were also abolished in Ampkα2-/- diabetic mice. These results suggest that AMPKα2 plays a central role in preventing DN. SFN prevents DN by the activation of lipid metabolism and NRF2-associated suppression of oxidative stress, and more important is that both of them are AMPKα2-dependent.

1981 Adaptation of Human Podocytes and Renal Proximal Tubule Epithelial Cell Lines to Physiological Oxygen Conditions

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In recent years it has become increasingly obvious that conventional 2D cultivation of cells cannot recapitulate their physiological environment, thereby altering the cellular phenotype. The latter limits the *in vitro* approaches usefulness in e.g. drug testing for adverse or off target effects due to limited or lack of predictivity. To overcome this issue research has increasingly focused on providing cells with conditions that mimic their physiological environment as closely as possible. Three factors of importance that need to be considered when designing *in vitro* experiments are cell-cell interactions, dimensionality and O_2 tension. Indeed, atmospheric O_2 (21%), at which nearly all *in vitro* experiments are carried out, massively exceeds physiologically normal O_2 *in vivo* tensions, e.g. 10% O_2 in the human kidney cortex, thus raising the question whether the excessive O_2 tensions lead to adaptive responses and thus to false negative and/or false positive responses upon exposure to nephrotoxicants. Two cell types critical for the structure and function of the kidney are proximal tubular epithelial cells (RPTEC) and podocytes (PODO). The objective of this work was to analyse the adaptation of RPTEC and PODO to physiological O_2 conditions and to characterise any phenotypical changes. Accordingly, RPTEC/TERT1 and PODO/TERT256 were cultured at 10% (PhysOx) and 21% O_2 (AtmOx). In order to support the polarity of the cells and thus allow for bidirectional transport, cells were cultured in transwells. Morphology, cell proliferation and cell metabolism (glucose consumption & lactate production) were assessed. Expression levels of markers for hypoxia and oxidative stress as well as structural markers and transporters were analysed at the mRNA level (RT-qPCR) and protein level (Western Blot, Immunofluorescence). Preliminary results show an increase of the HIF1α protein within 30 min at PhysOx suggesting an adaptive response. After adaptation to PhysOx for 14 days cells did not demonstrate a significant difference in cell proliferation, morphology or mRNA expression of oxidative stress and hypoxia markers when compared to their AtmOx counterparts. However, while RPTEC/TERT1 metabolism appeared to remain unchanged, a shift towards glycolysis in PODO/TERT256 metabolism was observed.

1982 Development of a Noninvasive, Multiplexed LC-MS Assay for the Quantification of Podocyte Injury Biomarkers in Human Urine

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The glomerulus is a specialized structure of the nephron that plays a critical role in blood filtration. An important cell type within the glomerulus required for appropriate filtration is the podocyte, which specialized foot processes interdigitate between adjacent podocytes to form the slit diaphragm, a filtration unit permeable to water and small molecules and impermeable to larger proteins. In glomerular diseases loss of podocytes compromises the ability to adequately filter blood and results in elevated glomerular filtration rate and proteinuria. Biomarkers of glomerular diseases in current use provide little information on mechanism or disease progression and thus kidney biopsy and subsequent histopathology is needed for disease diagnosis, which have limited throughput due to its invasiveness. Here, we present the method development of a non-invasive mechanism-specific biomarkers of podocyturia. We established a multiplexed immunoaffinity (IA) LC-MS assay to quantity four podocyte derived proteins, nephrin, podocin, podocalyxin and synaptopodin from clarified urine. Podocyte peptides suitable for LC-MS studies in urine were identified using proteomics and targeted MS investigations. Subsequently a high-sensitivity method was developed using a combination of online peptide IA and targeted MS. The achieved assay sensitivities were in the attomole/uL range with low limits of quantification (LLOQ) of 3.8 for nephrin, 12.1 for synaptopodin, 5.4 for podocin and 21 attomol/uL for podocalyxin. Assay inter- and intra-batch accuracy and reproducibility were determined in a three-day validation with typical CV and RE below 25%. Sample stability was demonstrated during 4-hour ice and RT and 3-day freeze/thaw stability studies. The assay performance was deemed to be suitable for clinical studies and the workflow will be implemented as an exploratory biomarker to evaluate podocyturia in glomerular disease patients in ongoing clinical trials.

1983 Modeling the Effects of Glomerular Dysfunction on the Proximal Tubule Using a 3D Microphysiological System

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Proximal tubule epithelial cells (PTECs) treated with albumin *in vitro* generate reactive oxygen species, secrete cytokines (e.g CCL2, IL-8), and produce fibrogenic molecules (e.g. TGFβ, fibronectin). This suggests that the proximal tubule may be a pathological mediator in the progression of chronic kidney disease, particularly in those with prominent glomerular dysfunction. Here, we treated a cell-based microfluidic model of the human renal proximal tubule with human serum or albumin to explore how altered glomerular filtrate composition can drive PTECs to acquire a maladaptive phenotype. Exposure of PTECs to 2% human serum increases the expression of marker of proliferation Ki-67 (KI-67) and secretion of kidney injury molecule 1 (KIM-1) and interleukin-6 (IL-6). Albumin treatment did not promote IL-6 secretion, but increased KIM-1 secretion in a donor-dependent manner. Co-treatment with cetuximab (anti-EGFR), tocilizumab (anti-IL-6R), or ruxolitinib (JAK1/2 inhibitor) did not inhibit serum-induced secretion of KIM-1 or IL-6, indicating that these processes may be independent of STAT3. Transcriptomic profiling by RNA-seq revealed that the expression of 820 genes were significantly affected by treatment with 2% human serum and Ingenuity Pathway Analysis™ identified the transforming growth factor beta and tumor necrosis factor alpha pathways as the two most likely upstream regulators mediating the transcriptional changes. Together, these data show PTECs become pro-inflammatory with serum treatment and indicate that this microphysiological system can be used to identify the signaling pathways that trigger a switch in PTEC phenotype.

1984 Development of a Model of Immune-Mediated Glomerular Injury in Cynomolgus Monkeys to Study Novel Kidney Safety Biomarkers

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Immune-mediated glomerular injury is more frequently observed with biotherapeutics as compared to small molecules which tend to produce tubular lesions in the kidney. A model of Cynomolgus monkeys treated with anti-Glomerular Basement Membrane (GBM) antiserum was developed to investigate new biomarkers of immune-mediated glomerular injury. The antiserum was produced from sheep immunized with GBM from Cynomolgus kidney cortex. A pilot study was conducted where monkeys were given 2 intravenous administrations of a first batch of anti-GBM antiserum at 5 mL/kg on Day 1 and 4 and then sacrificed on Day 10. Kidney microscopic findings consisted of severe glomerular alterations with mesangial proliferation/expansion in all animals, and minimal to mild tubular degeneration/necrosis and regeneration in 2/3 monkeys. Increases in microalbuminuria and proteinuria were observed from Day 4 in all animals, while increases in the urinary tubular biomarkers measured (N-acetyl-β-D-glucosaminidase (NAG), clusterin, retinol-binding protein 4 and osteopontin) were observed in only one animal on Day 4 and in all animals on Day 9. BUN and serum creatinine were increased in all animals on Day 9/10. Due to the tubular injury observed, a study was performed with lower doses to just induce the glomerular injury with 4 monkeys each treated with 1 intravenous injection of a second batch of anti-GBM antiserum at 2.0, 0.7, 0.2 mL/kg or the pre-immune serum at 2 mL/kg. The animal treated at 2 mL/kg was sacrificed on Day 10 as the predefined humane endpoints were reached, and the other animals were necropsied on Day 22. Minimal glomerular alterations and mesangial proliferation/expansion in the absence of tubular degeneration/necrosis were observed at 2.0 mL/kg with increases in microalbuminuria and proteinuria from Day 8. Minimal podocyte fusions were noted at electron microscopy at 0.7 mL/kg. Increases in microalbuminuria were observed at 0.7 and 0.2 mL/kg from Day 15 without increases in the tubular biomarkers measured (NAG, clusterin and cystatin C). These results indicate that monkeys treated with anti-GBM antiserum is a useful model of immune-mediated glomerulopathy which can be used to further investigate novel biomarkers of glomerular injury.

1985 4-methylpyrazole Protects against Acetaminophen-Induced Nephrotoxicity

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While acetaminophen (APAP) hepatotoxicity is the most common cause of acute liver failure in the United States, a significant percentage of APAP overdose patients also develop kidney injury. In contrast to APAP-induced liver injury where mechanisms of hepatotoxicity have been well studied, the molecular mechanisms involved in APAP-induced nephrotoxicity are relatively unknown. We have shown earlier that 4-methylpyrazole (4MP), a repurposed drug, protects against APAP-induced liver injury by inhibiting cytochrome P450-mediated generation of the reactive APAP metabolite, N-acetyl-p-benzoquinone imine (NAPQI). The mode of action has also been verified in human hepatocytes and in healthy volunteers. Since cytochrome P450 is also expressed in the kidney, this study explored protection by 4MP against APAP-induced nephrotoxicity, to determine if this intervention could serve a dual benefit in patients after an APAP overdose. Male C57BL/6J mice were treated with either 300 or 600 mg/kg APAP with or without 4MP for 2, 6 or 24 h, followed by measurement of APAP metabolism and tissue injury. Interestingly, levels of APAP and its non-oxidative metabolites were significantly higher in the kidney when compared to the liver at 2h after APAP. APAP-protein adducts were present in both tissues within 2h, but were absent in kidney mitochondria, unlike in the liver. While GSH depletion was seen in both tissues, activation of the MAP kinase JNK and its translocation to the mitochondria, which is a critical feature of APAP-induced liver injury, was absent in the kidney. Evaluation of tissue injury by histology, TUNEL staining, plasma BUN levels and Ngal (kidney injury marker) demonstrated a delayed kidney injury evident by 24h, notably at the 600mg/kg dose. Treatment with 4MP attenuated APAP metabolite generation, GSH depletion as well as kidney injury indicating its potential use in prevention of APAP-induced nephrotoxicity. In conclusion, since reactive metabolite formation seems to be common in both liver and kidney, 4MP mediated inhibition of Cyp2E1 protects against APAP-

induced nephrotoxicity, probably by increasing renal excretion of APAP and its sulfated metabolite. However, downstream mechanisms of APAP-induced nephrotoxicity seem distinct from the liver.

1986 Ochratoxin A Induces Epithelial to Mesenchymal Transition and Renal Fibrosis Through TGF- β/Smad2/3 and Wnt/β-catenin Signaling Pathways *In Vivo*

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Ochratoxin A (OTA) is a fungal toxin produced by fungi such as *Aspergillus app.* or *Penicillium spp*. OTA, which is present in various foods such as cereals, nuts, coffee beans, and beer, is a white, odorless toxin that has a very long half-life, is resistant to heat, and can accumulate when it enters the body. The main target organ that causes OTA toxicity is kidney, and it is known that epithelial to mesenchymal transition (EMT) and fibrosis are enhanced when the kidney is exposed to OTA for a long time. However, the mechanisms that cause these symptoms are not exactly known. Therefore, the aim of this study is to confirm the mechanism of OTA-induced EMT and fibrosis in mice kidneys. In this study, mice were orally administered for 12 w with various doses of OTA (200 and 1000 μg/kg body weight (B.W.)). In the group given to 1000 μg/kg B.W., blood urea nitrogen (BUN) and creatinine levels were increased 1.29-fold and 1.38-fold compared to the control group respectively. Also, fibrosis was also observed in kidney tissues. It also found that alpha-smooth muscle actin (α-SMA) and fibronectin were increased 5.65 and 2.48-folds and that E-cadherin decreased 0.26 fold compared to the control group by OTA in kidney tissues. In addition, the expression of TGF-β Receptor I, smad2/3 and β-catenin was also increased 3.71, 2.60, and 2.66-folds by OTA compared to the control group. These results examined that OTA induces EMT and renal fibrosis through Smad2/3 and β-catenin signaling pathway *in vivo*.

1987 Synergistic Toxicity between Ochratoxin A and Cadmium in Kidney Cells

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Ochratoxin A (OTA) is acknowledged as mycotoxin produced from several species of *penicillium* and *aspergillus* and frequently found in cereal grains such as rice and oat. Cadmium (Cd) is acknowledged as widespread toxic heavy metal and also found mainly in cereal grains. Therefore, there is a possibility that OTA and Cd may be ingested together and exposed in the body simultaneously. Although each individual toxicity of OTA and Cd have been studied, the combined toxicity has not been investigated. In this study, the combined cytotoxicity of OTA and Cd was investigated with human proximal tubule epithelial cells (HK-2 cells). HK-2 cells were exposed to OTA (25, 50, 100, 200, 400, and 800 nM), $CdCl_2$ (1.25, 2.5, 5, 10, 20, and 40 μM), and their mixture at 1:50 (OTA : $CdCl_2$) ratio for 48 h. The interaction effect of OTA and Cd on cytotoxicity was determined by the combination index method. Based on cell viability, compusyn analysis showed the interaction between OTA and Cd had synergistic effect, and the reactive oxygen species (ROS) also increased at concentration with the synergistic effect. Phase I reaction enzymes (CYP450), Phase II reaction enzyme (γ-glutamylcysteine synthetase (γ-GCS), heme oxygenase-1 (HO-1), and NADPH: quinone dehydrogenase-1 (NQO1)), and kidney injury-related marker, Kidney injury molecule 1 (KIM-1) were determined by quantitative real-time PCR. CYP450 enzymes (CYP1A1, CYP2E1), γ-GCS, HO-1, and NQO1 increased in combined treatment than in single treatment. KIM-1 also increased in combined treatment. Therefore, this study clearly showed that interaction between OTA and Cd have synergistic cytotoxicity in the kidney cells.

1988 Attenuation of 3,5-dibromoaniline Nephrotoxicity *In Vitro* by Antioxidants and Biotransformation Inhibitors

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Halogenated anilines are commonly used in the manufacture of a wide range of products, including drugs, agricultural agents, dyes and industrial products. Among the dibromoanilines, *in vitro* studies have shown that 3,5-dibromoaniline (3,5-DBA) is the most potent nephrotoxicant in rat renal cortical slices and isolated renal cortical cells (IRCC). The purpose of this study was to determine if free radicals and/or bioactivation played a role in 3,5-DBA nephrotoxicity *in vitro*. IRCC (3 mL 4.0 X 10^6 cells/ml) from male Fisher 344 rats were treated with DMSO (vehicle control), 0.5 mM 3,5-DBA, a pretreatment, or pretreatment plus 0.5 mM 3,5-DBA, followed by incubation for 60 min at 37°C. The pretreatments used in this study were: antioxidants [ascorbate (2.0 mM), glutathione (1.0 mM), N-acetyl-L-cysteine (2.0 mM), or α-tocopherol (1.0 mM)]; cytochrome P450 (CYP) general inhibitors [piperonyl butoxide (0.1 mM) or metyrapone (1.0 mM)]; a cyclooxygenase inhibitor [indomethacin (1.0 mM)]; a peroxidase inhibitor [mercaptosuccinate (1.0mM)]; and flavin monooxygenase (FMO) inhibitors [n-octylamine (2.0 mM) or methimazole (1.0 mM)]. Cytotoxicity was determined using lactate dehydrogenase (LDH) release analysis. LDH release was significantly increased from control levels by 0.5 mM 3,5-DBA treatment (~20-25% release of LDH). Among the antioxidants, ascorbate, glutathione and N-acetyl-L-cysteine attenuated 3,5-DBA cytotoxicity. 3,5-DBA cytotoxicity was also reduced by all biotransformation inhibitors except metyrapone. To explore CYP-induced bioactivation further, more specific inhibitors of CYP metabolism were used: oleandomycin (CYP3A4, 0.5 mM), omeprazole (CYP2C19, 0.01 mM) and DEDTCA (CYP2C/2E1, 0.1 mM). All three pretreatments attenuated 3,5-DBA. These results suggest that multiple enzyme systems may play a role in the bioactivation of 3,5-DBA to cytotoxic metabolites, and that reactive metabolites play a role in the mechanism of 3,5-DBA induced cell death. *Supported in part by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.*

1989 Kinetic Analysis of Trichloroethylene Glutathione Conjugates and the Effect of Renal Metabolites on Mitochondrial Respiration Using Cultured Human Renal Proximal Tubular Cells

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Trichloroethylene (TCE), a well-studied member of the halogenated alkenes, is produced in millions of kilos per year and poses a significant hazard to human health. Animal and *in vitro* studies have demonstrated that hepatic glutathione (GSH) conjugation and subsequent renal metabolism involving gamma glutamyl transferase (GGT) and cysteine conjugate β-lyase activity leads to the formation of a reactive thioketene, causing oxidative stress and mitochondrial injury. Here we re-examined the hepatic GSH-conjugation of TCE and the effects of GSH-conjugates of TCE on human renal proximal tubule cells. Utilising human liver fractions and recombinant human glutathione S-transferases we demonstrate the formation of 1,2-dichlorovinyl glutathione (1,2 DCVG). The human proximal tubular cell line RPTEC/TERT1 cells were differentiated and treated with 1,2 DCVG for 24 hours. A rapid and complete metabolism of the initial GSH-conjugate was observed within 2 hours, with a temporal transient increase of the respective glycine-cysteine conjugate, followed by the accumulation of the cysteine conjugate. The specific effects of the GSH-conjugate on mitochondrial respiration were quantified using the Seahorse bioanalyser. A decrease in mitochondrial reserve capacity was observed for 1,2-DCVG, but not in the presence of the β-lyase inhibitor aminooxyacetic acid (AOAA). This study better clarifies the molecular processes of TCE metabolism and toxicity in human renal cells demonstrating a clear relationship between metabolism and mitochondrial perturbation. *This work is funded by the EU's Horizon 2020 research and innovation program (EUToxRisk, grant agreement No 681002).*

1990 Resveratrol Protection of Cisplatin Renal Cytotoxicity and Mitochondrial Changes in Human Proximal Tubular Epithelial Cells

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Cisplatin is a cancer chemotherapy agent used in treating advanced testicular, ovarian and bladder cancer. Unfortunately, cisplatin is nephrotoxic. Resveratrol (RES) is a polyphenolic compound that promotes apoptosis in some cancer cell lines and reduces tumor size in clinical studies. Part of the mechanism for reducing cisplatin toxicity by RES may be mediated by preserving mitochondrial function and diminishing cisplatin induced oxidative stress. Cisplatin nephrotoxicity targets in humans, the proximal and distal tubules. HK-2 cells were selected for this study as they are a human renal proximal tubular epithelial cell line (HK-2). Our hypothesis is that RES will diminish mitochondrial impairment by cisplatin in HK-2 cells. HK-2 cells were plated for 48 h followed by a 1 h pretreated with RES or 1% DMSO (vehicle). Renal cells were co-incubated with cisplatin at a final concentration of 0-30 uM for 24 h. Viability was assessed using MTT and trypan blue exclusion cell counts. All studies were conducted a minimum of 3 independent experiments. Cells pretreated with 10 uM RES were protected from cisplatin cytotoxicity at 24 h. Cisplatin caused a concentration dependent decline in MTT viability after

24 h exposure to 0, 7.5 and 15 uM cisplatin. RES pretreatment for 1 h with 10 or 15 uM RES increased HK-2 viability relative to cells exposed only to RES Vehicle (DMSO). RES did not increase cell number as part of its protective mechanism. Mitochondrial function was decreased by cisplatin as evaluated using a Seahorse XFe Analyzer. Basal and maximal mitochondrial respiration were monitored in RES and cisplatin treated cells. Additional studies examined whether RES altered expression of mitochondrial complex proteins and markers of mitophagy. Western analysis detected an increase in protein carbonylation with 24 h cisplatin exposure which was reversed by RES. RES protects human proximal tubular epithelial cells from cisplatin cytotoxicity, preserves mitochondrial integrity and mitochondrial respiration. *Supported by NIH Grant INBRE 8P20GM103434 and a WV NASA Undergraduate Research Fellowship to MD and RM.*

1991 Dynamics of Transcriptional Responses in Cisplatin-Induced Kidney Injury Uncover Mechanistic Drivers of Toxic Renal Adverse Outcomes

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Drug induced kidney injury (DIKI) is still becoming one of the reason for high numbers of drug candidate attrition and drug withdrawal from the market. However, the mechanism underlining this adversity is still unclear hampering the method development for predicting DIKI. In particular, there is lack of temporal dose response information of cellular stress response data that could lead the way to a quantitative pathway-based hazard prediction of DIKI. As a proof-of-concept, we performed a systematic high throughput transcriptomic cisplatin temporal-concentration response study to investigate the gene expression profiles likely delineating the mechanisms of DIKI. Immortalized human proximal tubule cells (RPTEC/TERT1) were exposed to 10 concentration levels of cisplatin ranging from 0-50 µM. Samples were collected at 6 different time points (4, 8, 16, 24, 48, 72 hours) to capture both early and late responses. In parallel high content imaging experiments apical endpoints of toxicity were assessed. High throughput whole genome targeted RNAseq TempOSeq technology (~19000 genes) was used for transcriptional analysis. We observed robust transcriptional changes in a concentration and time dependent manner. We mapped the transcriptomics data to our previously established *in vivo* DIKI TXG-MAPr: a weighed gene correlation network analysis (WGCNA) approach to characterize and quantify gene-module activation based on gene co-regulation of DIKI transcriptomics studies. The TempOSeq data provided sufficient coverage for human to rat mapping. We revealed various gene-module modulation that followed a clear time and concentration dependency, including modules representing the DNA damage response. Systematic gene network modulation correlation analysis demonstrated that our *in vitro* results show high module correlation with the cisplatin exposed rat kidney prominently at the early time point indicating the similarity in acute responses between RPTEC/TERT1 and rat kidneys. We further provide insight in point-of-departure of transcription factor activation of the cisplatin responses and the quantitative relationships with the tipping points for onset of cytotoxicity. Altogether, our results and approaches bear a prospective insight to apply HTTr TempOSeq in combination with *in vivo* WGCNA approaches in establishing mechanism-based DIKI prediction. *The work was funded by the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking for the TransQST (grant agreement 116030) and eTRANSAFE (grant agreement 777365) projects. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA.*

1992 Effect of Gentamicin on Renal Proximal Tubule Epithelial Cell Transporters

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Human Renal Proximal Tubule Epithelial Cells (RPTEC) are an important *in vitro* tool for studying normal renal physiology, renal drug transporters, and nephrotoxicity. When culturing these primary cells, antibiotics are often added to prevent microbial contamination. However, antibiotics can adversely affect renal cells. In humans, nephrotoxicity is one of the most important side effects of the aminoglycoside antibiotic Gentamicin occurring in 10 - 25% of patients. It is therefore important to carefully select antibiotics with no or minimal impact on RPTEC function. This work characterizes the effects of two antibiotics on RPTEC *in vitro*: the frequently used cell culture antibiotic Gentamicin and the tetracycline Doxycycline. Specifically, we observe the effects of these antibiotics on the Organic Cation Transporter 2 (OCT2), the Organic Anion Transporter 3 (OAT3), and the Na+/K+ ATPase pump. To make a comprehensive assessment of the effects of the selected antibiotics on RPTEC, we isolated the renal cells with either Gentamicin or Doxycycline, followed by cryopreservation in the absence of either antibiotic. Subsequent culturing of these cells were done with or without Gentamicin in the culture medium. Immunocytochemistry (ICC) staining was used to determine the presence of OCT2, OAT3, and NA+/K+ ATPase. Results indicate that there is no visible difference in the effect of isolating RPTEC with Gentamicin or Doxycycline on Na+/K+ ATPase and OAT3 presence. This is also true of culturing the cells with or without Gentamicin. For OCT2, however, the transporter expression increases in Gentamicin isolated cells when compared to cells isolated with Doxycycline. When comparing cells cultured out of cryopreservation with Gentamicin to those that are cultured without antibiotics, the same observation is made: an increase in staining of the OCT2 transporter in the presence of Gentamicin. Interestingly, OCT2 has been found to contribute to the transport of Gentamicin into cells. Taken together, our data supports differences in the RPTEC phenotype when cells are exposed to Gentamicin or Doxycycline during cell isolation as well as when cells are cultured in the presence of Gentamicin versus no antibiotic. These findings provide guidance on optimal isolation methods and culture conditions for maintaining RPTEC function in drug transporter and nephrotoxicity studies with human renal proximal tubule epithelial cells.

1993 Acute *In Vitro* Nephrotoxicity of Three Brominated Flame Retardants in Renal Cells

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Brominated flame retardants (BFRs) are organohalogens commonly added to commercial products such as computers, electronics, textiles and furniture to reduce their flammability. BFRs have significant environmental persistence and are reported to be detected in human blood and breastmilk. In particular, tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), and polybrominated diphenyl ethers (PBDEs) occupy nearly 20 percent of the global flame retardant market and are suggested to have adverse effects on humans and wildlife. As such, the mechanisms of BFR-induced toxicity are actively being explored under the US EPA Toxic Substance Control Act. We previously determined the effects of TBBPA, HBCD, and BDE 47 on cell viability in rat (NRK 52E), adult human (HK-2 and RPTEC), and human embryonic (HEK-293) kidney cells after 48 hours, and demonstrated that these compounds induce concentration-dependent effects on MTT staining. Assessment of the mechanism of cell death using annexin V staining and propidium iodide (PI) staining showed that all three compounds induce apoptosis in these cells. We subsequently performed RNAseq and pathway analysis in these cells to identify key signaling pathways involved in the mechanism of BFR-induced toxicity. Furthermore, we explored how BFR nephrotoxicity impacts neighboring cells by treating human fibroblasts with conditioned media from BFR-treated kidney cells and measuring morphological, molecular, and functional changes in fibroblast activation using RT-PCR, wound healing and high content image analysis. Ultimately, these data will aid future studies concerning the molecular mechanisms of BFR toxicity in kidney cells and how this impacts kidney epithelia-fibroblast crosstalk using chronic exposure at environmentally relevant doses.

1994 Reduction of Colistin-Induced Kidney Damage in Mice by Co-administration of the Vitamin-Like Compound L-Carnitine

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Colistin is a polymixin antibiotic currently experiencing renewed clinical interest due to its efficacy in the treatment of multidrug resistant bacterial infections. The frequent onset of acute dose-dependent drug-induced kidney injury (DIKI), often leading to long-term renal damage, has limited its use and hampered adequate dosing regimens, leading to an increased risk of serum values below bacterial Minimal Inhibitory Concentrations (MIC) during treatment. The mechanism of colistin-induced renal toxicity is largely unknown but postulated to stem from its specific accumulation in the renal cortex through facilitated transport, coupled with mitochondrial toxicity and reactive oxygen species formation in proximal tubule cells after uptake during reabsorption. Several transport mechanisms for colistin in renal tissue have been elucidated, including facilitated transport over the peptide transporter 2 (Pept2) and endocytic uptake over megalin. We have recently shown that colistin is also a substrate for the carnitine/organic cation transporter 2 (Octn2/Slc22a5), expressed on the apical side of renal proximal tubule cells. Furthermore, we have shown that colistin treatment leads to structural damage at the site of the brush border membrane in proximal tubule cells, leading to loss of the sodium glucose transporter 2 (Sglt2), resulting in reduced reabsorption and

increased urinary excretion of glucose. In this study, a mouse model of subclinical DIKI was used to characterize colistin-induced kidney damage under co-administration with L-carnitine. We postulate that L-carnitine, sharing the transporter Octn2 with colistin, as well as eliciting positive effects on mitochondrial health per se, may be able to reduce colistin DIKI. Mice were treated with colistin sulfate (20mg/kg/day), L-carnitine (30 mg/kg/day), both, or PBS for seven days once per day intra peritoneally. Effects on expression, protein level, and transport capacity *ex vivo* of several proximal tubule transporters were analyzed. Assessment of tissue, urinary, and serum kidney damage markers was conducted. Treatment with L-carnitine alone did not impact kidney health but was able to protect against kidney damage in mice treated with both l-carnitine and colistin. We suggest using urinary glucose as a simple and cost-efficient marker of early kidney damage during colistin treatment and further support L-carnitine supplementation as a safe method of reducing DIKI caused by this antibiotic.

1995 Chrysin Ameliorates Cyclosporine A-Induced Renal Fibrosis by Inhibiting TGF-β_1-Induced Epithelial Mesenchymal Transition

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Cyclosporine A is a nephrotoxicant that causes fibrosis via induction of epithelial mesenchymal transition (EMT). The flavonoid chrysin (5,7-dihydroxyflavone) has been reported to have anti-fibrotic activity and has been shown to inhibit signaling pathways that are activated during EMT. This study investigated the nephroprotective role of chrysin in the prevention of CsA-induced renal fibrosis and aimed at determining the mechanism of inhibition of chrysin against CsA-induced EMT in proximal tubule cells. We have previously reported *in vivo*, treatment with chrysin and its analogue apigenin prevents CsA-induced renal dysfunction in Sprague Dawley rats as measured by the blood urea nitrogen (BUN), serum creatinine and creatinine clearance. Histopathological examination show that co-treatment with chrysin (10 or 50 mg/Kg) or apigenin (10 mg/Kg) inhibited CsA (50 mg/Kg) induced tubular vacuolization, cast formation within tubules, and increased collagen deposition: markers of tubulointerstitial fibrosis. *In vitro*, 25 μM chrysin significantly inhibited 4.2 μM CsA or 5 ng/ml TGF-β_1 -induced EMT in LLC-PK$_1$ cells evidenced by inhibition of cell migration, increased collagen expression, presence of mesenchymal markers like α-SMA and vimentin and decreased epithelial cell marker E-cadherin expression. Protein expression analysis further indicated that chrysin co-treatment decreased CsA-induced TGF-β_1 signaling pathways; decreasing Smad 3 phosphorylation leading to a subsequent decrease in Snail expression. Protein quantification also showed that chrysin inhibited the CsA-induced Akt/ GSK-3β pathway activation. Inhibition of both pathways resulted in a decreased cytosolic accumulation of β-catenin, a known trigger for EMT. In conclusion, flavonoids like chrysin offer protection against CsA-induced renal dysfunction and interstitial fibrosis. Chrysin was shown to inhibit CsA-induced TGF-β_1-dependent EMT in proximal tubule cells by modulation of Smad dependent and independent signaling pathways.

1996 Sodium-Dependent Dicarboxylate Transport of Diglycolic Acid in the Toxicity of Diethylene Glycol

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Diethylene glycol (DEG) is an industrial solvent, generally found in brake fluid and chafing fuels, that has been implicated in mass poisonings worldwide due to counterfeit, adulterated medicines. The predominant metabolites of DEG are 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA). Exposure to DEG causes kidney proximal tubular necrosis, as well as hepatotoxicity and a delayed peripheral neuropathy. DGA has been implicated as the metabolite responsible for the renal and hepatic toxicity observed during these poisonings and has been shown to accumulate greatly in the organs. Due to this accumulation, the mechanism of transport of DGA into kidney cells was of interest as a potential therapeutic target for this toxicity. DGA is structurally similar to succinate, which makes it a possible substrate for the sodium dependent dicarboxylate transporters (NADCs) in kidney cells. Pharmacological inhibition of NADC1 and NADC3 in human proximal tubule (HPT) cells showed a reduction in DGA accumulation. However, inhibitors were not specific enough to determine which of the transporters was more important to DGA accumulation. To answer this question, a genetic approach was utilized to knock down NADC1 and NADC3 activities in HPT cells via nucleofection with siRNA. The nucleofected cells were grown on membrane inserts and dosed apically or basolaterally with DGA. Knockdown was confirmed via qRT-PCR. Cell toxicity was measured using lactate dehydrogenase (LDH) release. Both NADC1 and NADC3 were knocked down in HPT cells when targeted with siRNA, but without much selectivity. Nevertheless, knockdown of NADC transporters reduced the resulting cytotoxicity of DGA, suggesting a decreased DGA uptake into cells. These results corroborate the pharmacological studies, but which transporter is the relevant one for therapeutic targeting has not yet been determined. *Supported by the American Chemistry Council.*

1997 An Adverse Outcome Pathway for Renal Tumors in Male Rats through Chemical Induction of α2u-globulin (α2u) Nephropathy (α2u-N)

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The National Research Council's vision of using adverse outcome pathways (AOPs) as a framework to assist with toxicity testing to meet regulatory demands of new and emerging chemicals has continued to gain traction since its initial release. The need to grow the current AOP knowledge base has gained urgency, with the US EPA's intent to eliminate animal toxicity testing by 2035. To meet these needs, an AOP for male rat renal tumors that occur through the ability of a chemical to induce α2u-N was organized based on extensive evidence of a specific mode of action (MoA) induced by multiple structurally diverse chemicals, including fuel additives (2,2,4-trimethylpentane), pesticides (1,4-dichlorobenzene), drugs (gabapentin), and solvents (decalin). This MoA is not relevant in humans, so it can be differentiated from other renal tumor MoAs using *in vitro* assays designed to measure the molecular initiating event (MIE) and/or key events (KEs) within an accepted AOP. Following identification and review of all relevant literature, including *in vivo* and *in vitro* studies, both the MIE and subsequent KEs were identified. The MIE is chemical binding to α2u, with KE1 being the inhibition of α2u catabolism due to chemical binding. This inhibition of α2u catabolism leads to its accumulation in the proximal tubule, resulting in the formation of protein droplets that crystalize (KE2). Exacerbated accumulation results in KE3 which is cytotoxicity, and compensatory cell proliferation. Atypical hyperplasia, along with lesions characteristic of protein accumulation, such as granular cast formation and linear mineralization, are considered to be KE4. With chronic exposure, a low incidence of renal adenoma and carcinoma development is seen in male rats. Based on the weight of evidence from the various evidence streams, the confidence in this AOP is high, and it could be used to assist with hazard identification in regulatory applications.

1998 Acute and Repeat-Dose Effects of a Panel of Electron Transport Chain (ETC) Inhibitors in Cultured Human Renal Proximal Tubule Cells

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Mitochondrial perturbations play a key role in the aetiology of several diseases and chemical-induced organ toxicities including acute and chronic renal disease. However, it still unclear how mitochondrial liabilities are sensed by cells and how the appropriate adaptive responses are triggered in terms of cellular signalling. To this end we utilised a differentiated human proximal tubule cell line (RPTEC/TERT1), with proven oxidative characteristics and challenged these cells to a panel of complex I, II, III electron transport chain (ECT) inhibitors. This chemical panel was developed within the EU-ToxRisk project. The effect of acute and repeat dose exposure was quantified utilising viability and biochemical assays, fluorescent microscopy and the Seahorse Bioanalyzer, which specifically monitors mitochondrial respiration by detecting changes in oxygen consumption rate/extracellular acidification (OCR/ECAR). In addition, we quantified the alterations of approx. 3000 genes using the TempO-Seq assay. Each of the assays utilised produced unique information, but most of the assays gave similar results to the potency of the compounds. The integration of the more specific assays allowed us to rank the compounds by potency, with Antimycin A and Rotenone always the most potent and Azoxystrobin the least. We saw no effect with complex II inhibitors. Transcriptomic analysis exhibited a good correlation between OCR/ECAR impact and the no. of significantly altered genes. However, complex I inhibition in general exhibit a more pronounced effect on the transcriptome then complex III inhibition. The most prominent pathway activated was the unfolded protein response. Again, there was no effect of complex II exposure on transcriptomic responses. Repeated exposures demonstrate increased toxicity. This study has explored several methods to quantify mitochondrial respiration and demonstrates that the combination of resazurin, supernatant lactate and OCR (particularity spare respiratory capacity) is optimum and that the RPTEC/TERT1 are a good model for such studies. This data and the optimised assays will be further utilised to develop a quantitative adverse outcome pathway for chemical induced renal mitochondrial disease includ-

ing Fanconi-like syndrome. *This work was part of the EU-ToxRisk project and received funding from the EU's Horizon 2020 programme under grant agreement No 681002.*

1999 Improved Phenotype of Human Proximal Tubule Epithelial Cells in a 3D Microfluidic Model

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Recently, renal *in vitro* models have advanced towards microfluidic three-dimensional (3D) cell cultures in which *in vivo* conditions are incorporated. Such culture platforms could enhance predictability of drug-drug interactions (DDI) and nephrotoxicity, meeting the demand of more predictable *in vitro* renal models in drug development. Here we showcase our 3D microfluidic human primary renal proximal tubule epithelial cell (PTEC) model. Fresh human kidney cortex was used to obtain PTECs, isolated following a collagenase digest and Percoll density gradient protocol. PTECs were cultured on Transwells® (Corning), referred to as aProximate™, or in a 3-lane OrganoPlate® (Mimetas), in which tubule structures were cultured on an extracellular matrix of collagen I (4 mg/mL, Bio-Techne) and exposed to fluid flow for up to 11 days. PTECs cultured in the OrganoPlate formed leak-tight tubules from day 7, as measured by leakage of Lucifer Yellow from the apical compartment. Next, cell viability, using WST-8 (Sigma), and cell membrane integrity, via cellular leakage of lactate dehydrogenase (LDH, CyQUANT™), were determined. Rate of growth of PTECs was higher in aProximate™ from day 7 to 11 (mean ± SEM; 250 ± 13%) compared to the OrganoPlate® (145 ± 6%). This was accompanied with a stark increase of LDH activity (660 ± 170%) in aProximate™, while a lower increase in LDH activity (450 ± 50%) was observed in the OrganoPlate®. Secretion of renal biomarkers (clusterin, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury marker-1 (KIM-1) into the supernatant was quantified using a custom multi-array technology plate (Meso Scale Developments). Levels of clusterin were higher in aProximate™ (341 ± 85%) as compared to the OrganoPlate® (181 ± 19%) at day 9. Secretion of NGAL increased in both cell culture platforms (237 ± 45% and 269 ± 23% in aProximate™ and OrganoPlate®, respectively) at day 9, while levels of KIM-1 were only found to be increased in aProximate™ (132 ± 31% and 92 ± 14% in aProximate™ and OrganoPlate®, respectively). These data showcased a 'healthier' PTEC phenotype when cultured in a 3D microfluidic model. Next steps include studying improved sensitivity towards DDI and nephrotoxicity, as well as incorporating other cell types to further increase biomimicry.

2000 Genomic Investigation of Thrombocytopenia in Monkeys with 2'-O-Methoxyethyl Antisense Oligonucleotide (2'-MOE ASO) Treatment

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Genetic influence on the incidence of 2'-MOE ASO-induced sporadic severe thrombocytopenia (TCP) (platelet counts 50<μL) in nonhuman primates (NHPs) is not well understood. Based on an integrated safety database of 102 distinct 2'-MOE ASOs from 61 studies and >2200 NHPs, severe TCP is observed at a low incidence (2%-4% at doses >5 mg/kg) in Asia-sourced cynomolgus monkeys (ACMs) (Henry SP, et al., NAT, 2017). Anecdotal evidence from various internal and external sources suggests that Mauritian-sourced cynomolgus monkeys (MCMs) have a markedly higher incidence of 2'-MOE ASO induced TCP compared to ACMs. The MCM population descended from small founder population introduced to the geographically isolated island of Mauritius in the 1500s (Sussman and Tattersall, Folia Primatology, 1986). The close-relatedness of MCMs may be advantageous for identifying genetic influences, if any, that play a role in ASO-induced TCP in NHPs. A study investigating subcutaneous 40mg/kg/wk dosing of ISIS 405879, a 2'-MOE ASO, induced severe TCP and mean platelet volume increase in 7/9 MCMs and 0/9 ACMs after 16 weeks of treatment. We performed exome sequencing of the 18 monkeys in this study along with banked samples from 52 ACMs treated with a variety of 2'-MOE ASOs picked in a 1:1 ratio of cases to controls. Multiple lines of evidence support an immune-mediated mechanism for severe TCP and it is possible that genetic variants could influence the immune response. The initial analysis of SNPs in genes that had been implicated in immune or drug-induced thrombocytopenia did not reveal an association with severe TCP. We noted 18 deletion variants (>100bp) in some NHPs compared to the *Macaca fascicularis* reference genome (MacFas5). These variants did not, however, segregate with TCP incidence and further analysis is needed is to draw a connection to TCP. Ongoing work includes strategies to aggressively limit the 450,000 typed SNPs to a handful of SNPs-of-interest to reduce multiple hypothesis testing *a priori*. Finally, we are doubling the number of monkey exomes from 70 to 140 to improve robustness in our genomic investigations.

2001 Mapping of a Highly Repetitive Enhancer Region within the IGH Gene Using Long-Read-Single Molecule Nanopore Sequencing

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Recently the cost of whole genome sequencing (WGS) has decreased while accuracy has increased. WGS of the human genome can be completed faster for cheaper utilizing "short read" sequencing (Illumina). Although these technologies provide highly accurate genotyping within simple regions, they lack the ability to map more complicated regions within the genome. Such regions include areas of high GC% bias, segments with large semi-palindromic sequences, and multiple tandem repeats. These regions make the *de novo* construction of more complete human genomes difficult. Single molecule technologies have provided a method to aid in the mapping of these difficult to sequence regions. The MinION nanopore sequencer (Oxford Nanopore Technologies) is one such technology that utilizes a bacterial protein pore to sequence intact strands of DNA. One benefit of this system is the ability to sequence large strands (> 100kb) without the need for PCR. Accuracy of single molecule sequencers is lower than that of short read sequencers. This has led to the development of algorithms that utilize long read data in tandem with the short read data to provide better *de novo* assembly of hard to map regions. Recently we induced edits in an enhancer (hs1.2) found within the 3'Immunoglobulin heavy chain gene regulatory region (3'IGHRR) in a human B cell line (CL-01). Both the regulatory region and hs1.2 enhancer are duplicated. The hs1.2 enhancer contains a 53bp invariant sequence (IS) that can be repeated one to four times in tandem. Flanking the hs1.2 enhancer are simple repeats and semi-palindromes. Because of these repeats, sequencing of this region cannot be done using short read sequencing, requiring the need for long read sequencing. To determine the exact edits we induced, we utilized MinION sequencing in conjunction with a protocol to obtain ultra-long reads. We made a reference genome of our cell line utilizing 1D MinION sequencing and performing analysis by hybridizing MinION reads and Illumina HiSeq X read. Because sequencing with the MinION does not require PCR amplification, we also have the ability to evaluate epigenetic modifications within the 3'IGHRR. Exact hs1.2 gene edits were determined through sequencing of amplicons capturing the full duplicated hs1.2 enhancer region. Long-read-single molecule sequencing using the MinION has been essential to determining the exact edits induced within the hs1.2 enhancer and to assemble a complete reference genome of our cell line.

2002 Evaluation of Connectivity Mapping Methods Using TempO-Seq Gene Expression Profiles

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Gene expression profiling has proven to be useful in identifying relationships between diseases, cell physiology, and therapeutics. Functional relationships underlying a disease, genetic perturbation, or treatment with a compound can be inferred by comparing the similarity of the experimentally derived "query" expression profile to a database of gene expression signatures linked to functions, diseases, or perturbations. A large-scale perturbation database that uses transcriptional expression data is The Connectivity Map (CMap). TempO-Seq® is a gene expression technology well suited for high throughput transcriptomics because it uses crude lysates, avoiding the need to extract or reverse transcribe RNA, permits pooling of many samples in a single sequencing run, and automated TempO-SeqR™ analysis software that does not require a bioinformatics expert to align the data, generate count tables, and identify differentially expressed genes or pathways. We evaluate the ability of TempO-Seq gene expression data to accurately identify signatures in the CMap database. We experimentally determined the TempO-Seq gene expression signatures for 4 compounds known to be included in the CMap database and asked how accurately the TempO-Seq signatures mapped to the CMap database signature for each. The 4 profiles were generated by treating MCF7 cells with cicloprox, genistein, sirolimus, and tanespimycin, with DMSO as a no treatment control. Signatures were generated by obtaining the log2fold change for each treatment versus control. These 4 signatures were mapped against the CMap build 02. Each signature was compared to the cmap database using three commonly used methods; the Kolmogrov-Smirnov method (KS), the extreme sum method (XSum), and the eXtreme cosine method (XCos). We find that for all 3 methods, cicloprox, sirolimus and tanespimycin are returned as

one of the top 5 results and genistein is in the top 20 results.We further evaluated the performance of each method by generating Receiver Operating Characteristics (ROC) curves. The areas under the ROC curves (AUCs) were calculated for each method. The xCos method (AUC =0.86) outperforms both the XSum (AUC=0.82) and KS (AUC=0.76) methods. Based on these results, we have incorporated the XCos method into the TempO-SeqR app, allowing for the user to align, perform QC analysis, obtain differential expression, fold changes and perform connectivity mapping of gene signatures using a single software application.

2003 Characterization of Reference RNAs and Lysates for Use as Gene Expression Controls

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Reference RNA samples are important positive controls for gene expression analysis. They provide a standard sample for quality control and a method of normalization between assay runs. The first widely adopted reference RNA samples were the MicroArray Quality Control (MAQC) reference A (Universal Human reference RNA, UHRR, Agilent) and B (FirstChoice® Human Brain Reference RNA, Ambion), described in papers published in Nat. Biotechnol. 2006, 24 (9) 1115-1169. MAQC A is a pool of total RNA extracted from 10 cell lines. MAQC B was a pool of total RNA from brain tissue of 23 donors which is no longer available. No other reference RNAs have been broadly adopted, nor is there any information about whether additional batches of these reference RNA's can be manufactured once they are exhausted, and little performance data is available. Most reference RNAs are complex mixtures of cells or tissues, with unclear manufacturing reproducibility. With EPA funding, we pursued the manufacture of reference RNAs that could be reproducibly manufactured in successive batches and which provide comparable performance to the MAQC references. We observed that when the contribution of accumulated cell lines to the total number of expressed genes was measured, the first cell line contributed ½ - ¾ of the genes, with each successive cell line providing diminishing additive contributions approaching an asymptote of 12,000-15,000 genes after incorporating 6-10 highly diverse cell lines. In contrast, we found that by treating cells with select compounds, we could achieve maximal numbers of expressed genes, differentially expressed genes, and differential expression range with only 3 cell lines and 2 compounds, pooled in different proportions, permitting repeatable culture and preparation of reference batches. We generated reference lysates first (EPA L1 and L2), then extracted the RNA for the matched reference RNA (EPA R1 and R2). We manufactured several batches to define repeatability and characterized performance; >5 logs dynamic expression range, >12,000 to 13,000 genes expressed by each, >11,000 expressed genes in common, with a range of 29 log2 fold change in differential expression between the samples of >5,000 genes (padj <0.05) at a sequencing depth of 8M reads. These results suggest that the EPA R1, R2, L1 and L2 reference controls should perform well as positive control samples, with particular utility in toxicology and drug discovery due to inclusion of cell responses to compound exposure.

2004 A Deep Learning Framework for Integrating Heterogeneous Data in Predictive Toxicology

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It remains a challenge to integrate multiple data sources for the prediction of toxicological endpoints. Utilizing the recent advancements in artificial intelligence, we developed a deep learning framework to integrate different data sources generated for the same objects, which produces more informative features for predictive model construction. In this study, we reported a deep neural network combined with an autoencoder, one of unsupervised learning architectures, for the purpose of integrative feature representation. Instead of restricting the input and output for the autoencoder to be the same, we allowed cross pairing between heterogeneous data sources. As a result, the generated features could represent the salient information from both platforms which would in turn improve the performance and robustness of the predictive model. We first demonstrated its utility in the SEquencing Quality Control (SEQC) neuroblastoma benchmark dataset, as our framework reached 0.854 in AUC for prediction patients' overall survival, which improved by 15% to 35% compared to previously established models. We then applied it to the prediction of Drug-Induced Liver Injury (DILI) potential via cell-line based assays of the drugs. The result showed our framework reached 0.261 of MCC in cross-validation, where the performance of other algorithms such as KNN, Random Forest and SVM were much lower, ranging from 0.094 to 0.161. In summary, we introduced a deep learning framework as a novel solution for integration of heterogeneous data to advance predictive toxicology.

2005 Mapping the Transcriptional Regulatory Landscape of Arsenic-Exposed Beta Cells

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Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia and pancreatic β-cell dysfunction. Laboratory and population studies have shown arsenite (iAs) or its methylated trivalent metabolite (MAs) can impair glucose stimulated insulin secretion (GSIS) by β-cells, but the underlying mechanisms remain uncharacterized. Transcriptional regulatory elements (TREs), including promoters and enhancers, which together control transcriptional regulation of gene expression, are important mediators of cellular response to environmental perturbations; however, they remain poorly understood in the context of arsenic exposure. Enhancers are classically defined as stretches of non-coding DNA that promote gene transcription by serving as binding sites for transcription factors, transcriptional coactivators, and RNA polymerase. We exposed rat insulinoma cells (INS-1 832/13) to non-cytotoxic concentrations of iAs (1 μM), MAs (0.5 μM) for 24 hours followed by RNA sequencing to investigate changes in gene expression. Matched chromatin run-on sequencing (ChRO-seq) was also performed to identify differentially active TREs in these cells. While both iAs and MAs significantly inhibited GSIS, high-throughput RNA sequencing revealed distinct gene expression profiles specific to each exposure. Specifically, we found that genes up-regulated by iAs are enriched in the NF-κB signaling pathway, a proinflammatory pathway previously associated with iAs exposure in other cell types. Genes downregulated by MAs are enriched in the regulatory network of FoxM1, a transcription factor (TF) necessary for the maintenance of adult β-cell mass, β-cell proliferation, and glucose homeostasis. ChRO-seq analysis revealed that more TREs are significantly altered by MAs exposure than by iAs (iAs: 9 up, 80 down; MAs: 312 up, 335 down). Exposure to iAs reduced the activity of TREs enriched in binding motifs for Rfx6 which regulates insulin transcription, content and secretion and for NeuroD1 in MAs-exposed cells, which controls insulin gene expression. Both exposures resulted in reduced activity of TREs enriched in binding motifs for Pdx1, a TF that is critical for establishing and maintaining β-cell identity. These altered TREs may represent an additional mechanistic link between arsenic exposure and disease.

2006 Utilizing FDALabel to Investigate Adverse Drug Reaction Patterns in Antidepressant Drug Labeling

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Depression, one of the most common mental illnesses in the US, is known to increase the risk of suicide, which is the tenth overall leading cause-of-death and second-leading cause-of-death among adolescents and young adults. As depression rates increase, so does the use of prescribed antidepressants, which can have potentially severe and sometimes life threating adverse drug reactions (ADRs), including suicide. FDA-approved drug labeling includes ADR information to help ensure the safe and effective use of prescribed drugs like antidepressants. This reliable source of information can be used to investigate and research potential ADRs to promote drug safety and public health. FDALabel is a searchable database containing drug labeling that can be used as a resource to locate important ADR information. DrugBank was used to identify 32 single active-ingredient drugs classified as antidepressants. A unique ingredient identifier for each of these drugs was used to query FDALabel for updated drug product labeling documents. Oracle SQL was used to extract ADR terms from different sections of these labeling documents. An ADR profile of the most frequently occurring terms was constructed to identify distribution patterns across the different labeling sections. All 32 antidepressant drugs identified had Boxed Warnings associated with the increased risk of suicidality in teens and young adults. Seven ADRs (*Depression, Completed suicide, Obsessive-compulsive disorder, Dizziness, Agitation, Mania, Seizure*) were identified among Boxed Warning, Warnings and Precautions, Pediatric Use, and Adverse Reactions sections of the labeling documents. Multiple ADR patterns were identified within the Warnings and Precautions sections. For example, Hepatic ADRs *Liver injury*, *Jaundice*, *Hepatotoxicity*, and *Hepatitis* occurred among two serotonin and norepinephrine reuptake inhibitor drugs, duloxetine hydrochloride (Cymbalta) and milnacipran hydrochloride (Savella). ADR patterns in antidepressant drugs could provide valuable information to assess risks and to promote safe and effective treatments for patients with depression. Here, information queried from drug labeling using FDALabel provided data for ADR analysis and pattern discovery in support of drug-safety research and public awareness of antidepressant drugs.

2007 How Complete Is Our Map of the Human Toxome? Functionally Enigmatic Genes as Targets of Chemical Perturbations

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The implementation of mechanism-based regulatory toxicology requires knowledge of the functions and pathways of target genes of chemicals. Incomplete information about genes in the human toxome can introduce biases to toxicological studies relying on annotation-based approaches. The purpose of this study was to identify the categories of gene annotation that are likely to be missed in chemical perturbation databases. We performed weighted gene co-expression network analysis (WGCNA) on human transcriptomic data sets, and correlated number of PubMed publications - an indication of annotation level - to various gene significance metrics. We showed that the amount of research was not correlated to the gene's biological importance. Moreover, these genes were not missing at random but reflected that our information about genes was gathered in a biased manner: poorly studied genes were more likely to be primate-specific and less likely to have a Mendelian inheritance pattern, tended to cluster in some biological processes and not others. Finally, many functionally enigmatic genes in diverse pathways - including primate specific genes - were potentially druggable or susceptible to chemical perturbations. In conclusion, underannotated genes would be invisible in a rodent model as well as in approaches based on prior pathway knowledge, underscoring the need for unsupervised analysis of human-relevant data sets in toxicology.

2008 A Machine-Learning Model Accurately Predicts TCDD-Induced Gene Expression

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The Aryl Hydrocarbon receptor (AhR) is a ligand-activated transcription factor that regulates a variety of genes to mediate the toxic actions of the persistent environmental contaminant 2 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). Joint analysis of AhR ChIP-Seq and RNA-Seq data from TCDD-treated MCF-7 cells showed that only 24 out of 218 up-regulated genes and one out of 87 down-regulated genes are AhR-bound in their proximal promoter regions. Several genes (228) show AHR binding in their promoter regions but are not differentially induced by TCDD. The underlying regulatory mechanisms for these observations are not clear. Epigenetic features including chromatin accessibility, DNA methylation, and histone modification have been reported to play important roles in controlling gene regulation. In this study, we have developed a machine learning model that predicts the gene expression (up-regulated, down-regulated, or unperturbed) in TCDD-treated cells based on histone modifications and chromatin accessibility, AHR ChIP-Seq data as well as DNA sequences in the gene promoter regions. Our predictive model is interpretable, clarifying the complex interactions among these multiple genomic and epigenomic features regulating TCDD-induced gene expression. The model will be validated with RNA-Seq data from TCDD-treated HepG2 cells and primary human hepatocytes. This work will: i) Improve our understanding of AHR-mediated gene regulatory networks and combinatorial gene regulatory effects of epigenetic modifications; and ii) provide a quantitative framework to predict inducible gene expression upon exposure to non-genotoxic chemicals.

2009 Prediction of Genome-Wide Aryl Hydrocarbon Receptor Binding from DNA Sequence, Chromatin Accessibility, and Histone Modifications

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The Aryl Hydrocarbon Receptor (AhR) is an inducible transcription factor (TF) whose ligands include the potent environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AhR regulates genes involved in a variety of physiological and developmental processes, and its dysregulation has been linked to multiple adverse outcomes like liver toxicity and immune suppression. Mapping of AhR-mediated gene regulatory networks is critical for improved understanding of the intracellular events that lead to tissue-specific adverse health effects upon exposure to TCDD-like chemicals. However, the identity of *in vivo* AhR binding sites is unknown in most human tissues; and computational prediction of genome-wide binding sites and identification of target genes for the AhR and other environmentally induced TFs remains a formidable challenge. In this study, we applied XGBoost, a supervised machine learning model, to predict DNA binding sites of the AhR in MCF-7 breast cancer cells and human hepatocytes. We trained the model on DNA sequence features and epigenetic marks including chromatin accessibility and histone modifications (HMs). Our results showed that a combination of DNA sequence and specific epigenetic marks can predict AhR binding sites with high accuracy. Comparison of the HM patterns surrounding bound vs. unbound DNA sequences centered on the canonical 5′-GCGTG-3′ AhR binding motif showed significant differences in HMs both within specific tissues and across tissues. We also found the relationship between epigenetic marks and AhR binding affinity to be region-specific across the genome. Our work provides a robust modeling framework to predict binding sites for the AhR and other ligand-inducible transcription factors in a tissue-specific manner.

2010 How Similar among Different Toxicogenomics Study Designs for Liver?

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Toxicogenomics (TGx) is an important tool to gain an enhanced understanding of toxicity at the molecular level. A broad ranges of TGx study design has been reported, some based on the existing animal models (e.g., one-day short-term assay or repeated dosing for 28 days) and the other applied *in vitro* systems (e.g., cell lines from rat, humans and cancer). A question is naturally rasied: how similar among different TGx study designs? In fact, this question can be asked in many different ways: (1) is a one-day *in vivo* short-term assay able to replace the 28-day standard and expensive toxicological assay? (2) are some biological processes more conservative across different preclinical testing systems than others? (3) do these preclinical testing systems have the similar resolution in differentiating drugs by their therapeutic uses? (4) Is it possible for *in vitro* to *in vivo* extrapolation? And (5) can genomic profiles from a cancel line predict drug-induced liver injury? These questions were explored using several large genomics datasets including Open TG-GATEs and L1000 for assesing drug-induced livery injury with proposed PRank methodology. For rat *in vivo*, one-day single dosing is very similar to the 28-day repeated dosing (score=0.9). A better IVIVE concordance was obtained for rat primary hepatocytes (score =0.71) than for human primary hepatocytes (score =0.58), indicating species difference playing a critical role in IVIVE. When limiting the analysis to only these drugs causing severe DILI, the IVIVE concordances were improved for both rats (from 0.71 to 0.76) and humans (from 0.58 to 0.62). Furthermore, we observed an encouraging similarity between HL60 and human primary hepatocytes (score = 0.70), suggesting the two cellular assays could be potentially interchangeable. In conclusion, this study provided valuable information for selecting the 'fit-for-purpose' TGx assay for drug safety evaluation.

2011 Transcriptional Comparison of Liver Cells to Reveal Biological Networks and Pathways Differentially Regulated for Each *In Vitro* Liver Model Systems

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Hepatic injury by drugs continues to be a major concern in drug development. There is a requirement for predictive and mechanistically informative models to aid the risk assessment. In the early phase of drug development, *in vitro* cell-based assays were proposed as potential solutions to predict drug induced liver injury (DILI). Although several types of liver cells are often used for developing such *in vitro* assays, it is not understood what biological networks and pathways are similar or differ between each *in vitro* liver model and human liver tissue. In this study, transcriptome analysis was performed with human liver tissues, human hepatocytes freshly isolated from liver-humanized mice (PXB-cells), cryopreserved primary human hepatocytes, HepaRG, and HepG2 cell lines by using RNA-seq to understand the gene expression characteristics of each liver model. We used the Weighted Gene Co-expression Network Analysis (WGCNA) approach to reconstruct gene co-expression networks for human liver model cells and liver tissues. This analysis generated 26 modules for 14,826 transcripts. The result suggested that genes related to FXR/RXR, PXR/RXR, and LXR/RXR activation pathways were differentially regulated among the liver models. Those genes showed higher expression in human liver tissues and less expression in HepG2 and HepaRG cell lines. Human primary hepatocytes and PXB-cells showed a gradual increase in those genes over time of culture. Because nuclear receptors like PXR, RXR, and LXR play a key role in the transcriptional control of critical steps of hepatobiliary transport and phase I/II metabolism of endo- and xenobiotics such as bile acids and drugs, our result indicated that primary hepatocytes and PXB-cells would be better models to capture cholestatic liver injury than the other

liver cell lines. When we developed an *in vitro* assay which could quantitate drug-induced bile acid efflux transport inhibition with the liver model cells by using synthesized fluorescent analogue of natural bile acid cholylglycine (cholyl-L-lysyl-fluorescein: CLF), PXB-cells detected cyclosporine A induced dose dependent CLF efflux inhibition with higher sensitivity than HepaRG. By understanding biological network and pathway differences among the liver model cells to develop *in vitro* mechanistic assays for liver toxicity, we can improve the predictability of DILI.

PS 2012 FAIRtox: A Web Application to Promote Data FAIRness at the Michigan State University Superfund Research Center

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The Michigan State University (MSU) Superfund Research Center (SRC) has produced vast amounts of data characterizing the mechanisms and impact of exposure to aryl hydrocarbon receptor (AHR) ligands. These data, ranging from single-endpoint *in vitro* assays to *in vivo* omics, are a valuable resource to the research community, yet their reuse is hampered by limitations in existing repositories and resources for re-analysis and integration. Findability, Accessibility, Interoperability, and Reusability (FAIR) data principles have been developed to maximize the impact of publicly funded research data. To further advance the FAIRness of MSU SRC data, we have developed a web application, FAIRtox, to access, query, integrate, and reuse SRC data. Currently, FAIRtox manages microarray and RNA-sequencing datasets for TCDD, PCB126, or PCB77 exposures in mouse, rat, and human *in vivo* and *in vitro* models with comprehensive metadata that provide study design details and context to support independent analysis and interpretation. Querying and analysis tools within FAIRtox enable comparisons and integration of deposited datasets for dose-response, time-course, and circadian regulated study designs to further characterize how diurnal rhythm and treatment duration impact hepatic gene expression. Furthermore, basal expression levels across multiple samples provides additional insight into interactions between sex, diurnal rhythm, and toxicity. FAIRtox serves as a centralized resource to access the diverse data types generated by the MSU SRC, along with meta-data, and tracked to persistent locations in publicly available repositories such as the Gene Expression Omnibus (GEO) and Dataverse. FAIRtox is developed using R Shiny (RStudio) to leverage the various packages to process and analyze various biological data types. Distribution of the web-application through code-sharing repositories, and packaged as a Docker Image, will make the tools and data publicly available. The goal of FAIRtox is to improve data sharing, reuse, and reproducibility to further elucidate mechanisms of toxicity and support the human risk assessment of chemicals of concern. *Funded by the Superfund Research Program P42ES04911.*

PS 2013 Machine-Learning Prediction of Cyanobacterial Toxin (Microcystin) Toxicodynamics in Humans

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Blue algae, cyanobacterial blooms and the concomitant presence of highly toxic cyanotoxins in surface waters used for drinking water and recreational purposes are a serious recurring health hazard. Among the most frequently reported cyanotoxins microcystins (MC) represent a family of cyclic peptides with approx. 250 congeners demonstrated to be harmful to human health due to their ability to inhibit ser/thr-proteinphosphatases (PPP). Indeed, the Caruaru incident in Brazil in 1996, where a high number of dialysis patients died following exposure to MC contaminated water during dialysis, or the most recent closing of the drinking water supply for the inhabitants of Toledo, Ohio, USA, resulting from recurrent *Microcystis aeruginosa* blooms in Lake Erie, dramatically emphasize the health hazards present. Despite the latter and recent efforts by WHO to improve the basis for human risk assessment and management all current hazard and risk assessments (HA/RA) are based on data of MC-congener (MC-LR), i.e. 1 of the 250 known MC congeners, only. Indeed, the fact that no MC-LR but rather a mixture of several MC congeners was deemed causal in the Caruaru incident in Brazil, only compounds the present situation with regard to HA/RA. MC congener structural diversity is a challenge for the risk assessment of these toxins, especially as in addition the several different PPPs have to be included in the HA/RA. Consequently, the inhibition of PPP1, PPP2A and PPP5 was determined with 18 structurally different MC and demonstrated MC congener dependent inhibition activity and a lower susceptibility of PPP5 to inhibition than PPP1 and PPP2A. The latter data were employed to train a machine learning algorithm that should allow prediction of PPP inhibition (toxicity) based on MCs 2D chemical structure. PPP inhibition IC_{50} values were classified in toxicity classes and three machine learning models were used to predict the toxicity class, resulting in 80-90% correct predictions.

PS 2014 A Predictive Classifier to Distinguish Nuclear Receptor Binding Sites Involved in Hepatic Cytochrome P450 Gene Regulation

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The liver is the central processing hub of xenobiotic chemicals and drugs in the human body. A handful of Cytochrome P450 (CYP) enzymes: CYP3A4, CYP2C9, CYP2C8, CYP2E1, CYP1A2, CYP2A6, CYP2D6, CYP2B6, CYP2C19, CYP3A5, CYP2J2, CYP1A1 and CYP1B1, account for more than 90% of liver xenobiotic metabolism. Expression of CYP genes is regulated by a network of hepatic nuclear receptors including hepatocyte nuclear factor-4-alpha (HNF4α), peroxisome proliferating factor alpha (PPAR), and retinoic X receptor (RXR). Recently assembled publicly available functional genomic data sets have allowed for a systems approach to understanding genome-wide regulation of toxicological responses. Using the CYP regulatory network as a model, we collected relevant epigenomic datasets. Visualizations of these epigenomic datasets reveal overlaps between transcription factor binding sites, DNaseI hypersensitivity sites, contextual histone modifications and pioneer factor binding in liver cells. These visualizations suggest a generalizable and predictable set of features for nuclear receptor binding sites. Using ChIP-Seq peaks, characterized binding sites and their flanking sequences using motif scanning. We utilized a combination of dimensionality reduction algorithms to graphically represent sequence and epigenetic differences among nuclear receptor binding regions. Using statistically and visually differential features in our dataset we developed machine learning classifiers to annotate putative response elements for hepatic nuclear receptors. This work will provide a dynamic framework to assemble gene regulatory networks underlying biological responses to a broad class of non-gentoxic chemicals.

PS 2015 Prediction and Analysis of miRNA Targets for Genes Conferring Risk to Breast Cancer Susceptibility

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Breast cancer is a leading cause of death among women worldwide. The constant acquisition of genetic and epigenetic alterations leading to tumor heterogeneity causing a major problem in designing effective therapies for most cases. Previous studies showed that the genes *BRCA1*, *BRCA2*, *PTEN,* and *TP53* are responsible for most of the inherited syndromes in breast cancer. MicroRNAs, on the other hand, are known to show a regulatory role in breast cancer. In the present study, we aimed to predict and analyze miRNA targets for the genes conferring high risk to breast cancer susceptibility using computational approaches. To this end, we have identified the miRNAs targeting the genes *BRCA1*, *BRCA2*, *PTEN,* and *TP53* across the 3′ UTR, 5′UTR and CDS regions using miRWalk, miRDB, and Targetscan databases. Our results showed miRNAs hsa-miR-4524b-3p, hsa-miR-384, hsa-miR-4524b-3p, hsa-miR-384, hsa-miR-4684-5p for *BRCA1;* hsa-miR-186-5p, hsa-miR-450a-1-3p, hsa-miR-450b-3p, hsa-miR-3176, hsa-miR-4432 for *BRCA2*; hsa-miR-7152-3p for *PTEN* and hsa-miR-138-5p, hsa-miR-587 for *TP53* were predicted as top five significantly aligned miRNAs at a p-vlaue of < 0.0001 in the CDS region. Further results showed enrichment of Gene ontology (GO) terms dna damage response, positive regulation of cell cycle arrest, apoptotic process, dna repair, cell cycle in *BRCA1*, multicellular organism growth, nucleotide excision repair, regulation of cytokinesis, histone h4 acetylation in *BRCA2*, t cell receptor signaling pathway, regulation of protein stability, protein stabilization, cell proliferation, cell migration in *PTEN* and base excision repair, cell proliferation, cell cycle arrest, cell aging in *TP53*. In conclusion, results from our study indicate that these miRNAs can be used as potential biomarkers for early cancer diagnosis and therapy.

2016 Discrepancy in the Safety Information of the US FDA and EMA Package Insert Has a Clinical Impact

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Ensuring the safe use of medicine is a major responsibility of the US Food and Drug Administration (FDA), which is listed as a priority in the FDA's Strategic Policy Roadmap. Hepatic adverse reaction is a main concern in drug safety and a leading cause of drugs being withdrawn from the market. To minimize the occurrence of the adverse event, the FDA issues a package insert (or drug label) for each approved drug, which provides evidence-based safety information as a reference for both healthcare professionals and patients. Regulatory agencies such as the FDA and its counterpart in Europe, European Medicines Agency (EMA) independently review and approve package inserts, which results in some degree of discrepancy in the safety features of the package insert. This raises critical concerns that such discrepancy could be misleading to physicians and thus increases the risk of adverse events. Here, we performed a systematic comparison of the hepatic safety information in the FDA's and EMA's package insert by using two sets of classification schemes. The labeling discrepancy regarding hepatic adverse reactions and clinical monitoring requirement was estimated to be 22-32% and 31% respectively. Much higher discrepancy rates were observed in contraindications (74%) and dose adjustment recommendations (67%) for pre-existing liver diseases. Notably, based on the analysis of the FDA's global adverse events report data, serious hepatic adverse events were found to be more commonly associated with drugs that have labeling discrepancy than those without labeling discrepancy. Therefore, the difference in risk perception and management between the two agencies has an impact on the patient clinical outcomes. The labeling discrepancy is mainly due to the differences between the FDA's and EMA's Guidelines, which are followed in making the package insert. This is not expected to be fully resolved considering the nature of the two agencies but could be at least minimized. Efforts in enhancing international communications and collaborations would be appreciated for the improvement of public's health.

2017 Predicted Genetic Networks Associated with Isocyanate Biomarkers of Exposure and with Susceptibility to Isocyanate-Induced Asthma

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Isocyanates in polyurethane paint and spray-foam insulation are known respiratory and skin sensitizers of global concern because they are a leading cause of occupational asthma. However, only a subset of about 5 - 15% of isocyanate exposed workers develop asthma. Thus, we hypothesize that inter-individual genetic variance may influence biomarkers of exposure and asthma susceptibility. We investigated genetic markers associated with variability in isocyanate biomarker levels and reviewed published data reported on isocyanate-induced asthma susceptibility markers in exposed workers. We quantified personal exposure and biomarker levels and performed genome-wide associations using mononuclear blood cell DNA of 33 male spray-painters. Using logistic regression and controlling for inhalation and skin exposure as covariates, we identified 20 single nucleotide polymorphisms (SNPs) associated with differences in isocyanate biomarker levels. These SNPs were within or proximal to 16 genes: ACVR1, ACVR1C, FHOD3, GDNF, RIMS1, C2orf71, DGKB, ETV1, BMPR1B, CNTN3, GRK5, KCNQ5, L3MBTL4, PDZRN3, PHF2, and SALL1. To identify genes associated with isocyanate toxicity, we systematically searched the literature for genetic markers that were over-represented in individuals with isocyanate-induced asthma. We found 166 non-duplicate publications, of which 24 contained relevant human data that were included in our analysis. Multiple studies reported significant markers within 17 genes: CDH17, CTNNA3, GSTM1, GSTM3, GSTP1, GSTT1, HLA-A, HLA-B, HLA-C, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRB1, NAT1, NAT2, TNF-α, and ZBTB16. Gene ontologies associated with these combined gene sets were evaluated using GeneMANIA, a gene ontology enrichment database that predicts network associations. Our analysis shows interactive networks that include cell-cell adhesion, transcription modulation, regulation of ossification, and immune cell activation. These predicted genetic networks can help direct future research on allergic airway sensitization by isocyanates and development of better at-risk worker protections.

2018 New Potential Targets for Phosphodiesterase-5 Inhibitors

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Male sexual enhancement supplements (MSES) are prescribed for erectile dysfunction and premature ejaculation. Most commercially available MSES are derived from sildenafil, a known phosphodiesterase type 5 (PDE5) inhibitor. In many countries, these drugs are sold to the public without a prescription, becoming a concern due to several adverse effects for which there is little information on their biochemical mechanisms. The aim of this work was to identify plausible human pharmacological targets for PDE5 inhibitors utilizing a computational approach. Optimized 3D structures of ten common PDE5 inhibitors were used as input ligands to search for potential target identification employing PharmMapper server. Best protein targets were subsequently examined by using protein-ligand docking methods with AutoDock Vina program. Results showed protein-ligand complexes with greater promissory scores (kcal/mol) were obtained for aromatase (PDB, 3EQM)/taladafil (-10.3); ribosyldihydronicotinamide dehydrogenase (3GAM)/aildenafil (-10.1) and mitogen activated protein kinase-10 (1PMV)/sildenafil (-9.6). These data suggest PDE5 inhibitors have the potential to modulate different biochemical processes, including hormone homeostasis, coagulation, and neuronal proliferation, differentiation, migration and programmed cell death, among others. Molecular re-docking experiments demonstrated the reliability of the docking protocols (RMSD <0.8 Å). Biological data for 47 PDE5 inhibitors (PubChem BioAssays, AID: 446781) displayed good correlation with the binding affinity from AutoDock Vina (r=0.640, p<0.001). These findings should encourage additional toxicological evaluations to better guarantee the safety use of MSES. *Colciencias-Unicartagena (528, 2011).*

2019 US FDA-Approved Drug Labeling: A Rich Resource for Pharmacogenomic Information in the Application of Precision Medicine

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Pharmacogenomics (PGx) is the study of how genomics and genetic variants affect drug response. FDA-approved prescription drug labeling includes important drug safety and efficacy information needed to prescribe drugs appropriately. Drug Labeling is a critical foundation for evidence-based medicine and include PGx information. However, for many drugs, PGx information is presented among different sections of labeling, making it challenging for the healthcare community to effectively identify and utilize the information. We summarized drug labeling PGx information obtained by searching FDALabel (https://nctr-crs.fda.gov/fdalabel/), a publicly available database developed by FDA/NCTR. A total of 261 prescription drug labeling documents were identified by querying 75 known biomarkers. The most frequently observed biomarkers by drug counts occurred in the order of CYP2D6 (66) > G6PD (39) > CYP2C19 (22) and covered a broad range of therapeutic classes (e.g., Psychiatry, Cardiology, Oncology, Endocrinology). In addition, four categories of applications were discussed according to information provided within the labeling: (1) Indication - useful for identifying responders and non-responders, (2) Safety - predicting patients at greatest risk for drug-induced adverse drug reactions, (3) Dosing - forecasting precision dosage adjustments for patient subgroups defined by PGx, and (4) Information - descriptive clinical pharmacology related to pharmacokinetics or pharmacodynamic information. This information could enhance the mechanistic understanding and clinical utility of PGx information in drug labeling and enable bench-to-bedside implementation of individualization of drug therapy for patients. In summary, this study provides a comprehensive assessment of PGx data in FDA drug labeling and offers researchers, regulators, drug developers, and physicians, an effective and efficient way to use the information to advance precision medicine.

2020 Using ANOVA-Based Clustering to Evaluate Chemical Response Patterns in Quantitative High-Throughput Screening Data

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Quantitative high throughput screening (qHTS) experiments can simultaneously test thousands of chemicals for potentially adverse effects by generating concentration-response data for each chemical. However, artifacts and influential experimental factors may lead to distinct clusters of response pat-

terns within a single tested chemical. We describe a novel approach based on analysis of variance (ANOVA) to reliably identify separate response clusters within each chemical. Correlations between 7,229 chemical response patterns were assessed across 43 bioassay data sets from phase II of the Tox21 collaboration. The fraction of chemicals in an experiment with consistent response patterns (f_1) and the fraction with inconsistent response patterns (f_2) describe the extent of assay response and provide a measure of assay quality. Here, no relationship between f_1 and assay noise was detected across assay readouts, but a significant association between f_2 and assay noise was found (spearman $p < 0.01$). The proportion of hits with consistent responses among all detectable hits (r_1) was largest when there was only one supplier, one site of preparation or one compound purity per chemical. For example, in agonist assays the median r_1 was larger when chemicals were represented by only one supplier (median $r_1 = 0.25$) compared to cases in which chemicals were represented by two different suppliers (median $r_1 = 0.11$) or three different suppliers (median $r_1 = 0.07$). When performing a large-scale correlation analysis between all 72 assay readouts generated in the 43 bioassay data sets, most agonist assay readouts were positively correlated with each other in a single "activator" correlation block. Similarly, antagonist and cell viability assay readouts were positively correlated with each other in a separate "inhibitor" correlation block. However, response patterns from 8 out of 24 agonist assay readouts appeared in the inhibitor correlation block, with 5 out of these 8 readouts exhibiting negative overall mean responses. This correlation between bioassay responses from agonist assays and antagonist or cell viability assays may be due to widespread cytotoxicity effects. Overall, these results suggest that correlation analyses between bioassay response patterns can be used to assess bioassay quality and flag specific experiments for unexpected chemical response signatures.

2021 Integration of Transcriptomic Point-of-Departure Metrics into the MoAviz Visualization Framework

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Gene expression profiling is emerging as a viable way to evaluate mode of action and points of departure and has the potential to drastically reduce testing costs and product development time. The first steps of using transcriptional responses as a basis of safety assessment are being taken. However, several important considerations remain unresolved, including how to translate expression changes into adverse outcome pathways or other definitions of mode of action, and the best manner with which to summarize gene expression data into a point of departure. Recently we developed an interactive browser application, MoAviz, to facilitate the examination of gene expression data across dose and time for mode of action studies of chemical perturbation for 204 compounds (spanning 290 million gene expression change values). We used MoAviz to quantitatively compare pathway-level transcriptomic signatures across compounds with well-known modes of action, and across different model systems, providing the groundwork for performing "biological read-across" between compounds based on their transcriptomic fingerprints. We evaluated the extent to which gene expression changes from in-life exposures could be associated with mode of action by developing a novel similarity index—the Modified Jaccard Index (MJI)—that provides a quantitative description of genomic pathway similarity. While typical compound-compound similarity is low (MJI = 0.026), clustering of the TG-GATES compounds identifies groups of similar compounds. Some clusters aggregated compounds with known similar modes of action, including PPARα agonists (MJI = 0.330) and NSAIDs (MJI = 0.327). We continue to extend the MoAviz interface and database by incorporating whole transcriptome benchmark dose analyses and point of departure (POD) summary, including the command line modeling features of the BMDExpress2 software. This integration will include statistical pre-filtering of transcriptomic gene expression data, dose response modeling of individual genes, ontology over-representation of genes, and POD summary based on current proposed best practices for gene-based and pathway-based derivation of POD. By combining mode of action and POD tools in an interactive interface, MoAviz will facilitate the use of transcriptomics data over a variety of chemical safety contexts.

2022 Binding Proteins for Curcumin and Bixin

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Curcumin and bixin are natural pigments obtained from *Curcuma longa* and *Bixa orellana*, respectively. These chemicals are widely used in cosmetic products and exhibit diverse pharmacological properties that make them promising cosmeceuticals. Although both natural products protect against toxicity induced by diverse xenobiotics, little is known about their interaction with signaling transduction pathways. The objective of this work was to find binding proteins for these two molecules, aiming to link them to their reported biological activities. A total of 977 human proteins were evaluated as potential targets for these two chemicals through docking-based virtual screening. *In silico* molecular protein-ligand docking protocols were performed using AutoDock Vina, and proteins with best binding affinity values were further uploaded to public databases in order to reveal potential underlying pharmacological mechanisms. Protein-protein interaction networks were constructed using String server and Cytoscape, based on information provided by KEGG, PDB and Uniprot databases. Proteins complexes with best docking binding affinities (kcal/mol) included 4EY7-curcumin (-11.4), 3PM0-curcumin (-10.7), 1HK4-bixin (-10.5) and 4EY7-bixin (-10.4). The results showed curcumin and bixin have the potential to modulate signaling pathways related to estrogen receptor, steroid hormone biosynthesis, PI3K-Akt, cancer, and aminoacid metabolism, among others. Findings presented here may provide information that will improve our understanding of the pharmacological mechanisms of bixin and curcumin, and will pave the way for additional therapies employing these cosmeceuticals. *COLCIENCIAS-UNICARTAGENA, FP 44842-212-2018.*

2023 Does Transcriptional Response Add Value to Predicting Drug-Induced Liver Injury?

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Drug induced liver injury (DILI) is caused by widely diverse mechanisms that can be specific to various drug classes. Variable types of injury (hepatocellular, cholestatic, steatosis) have led to the withdrawal or black box warnings for many efficacious compounds. DILI risk annotation for compounds is complicated and controversial. The federal drug administration has established a set of suggested risk classification groups, leading to four categories: most, less, no and ambiguous DILI concern. We tested 69 drugs from the most and no DILI concern categories in a novel *in vitro* model comprised of the HepG2 cell line and human peripheral blood monocytes (PBMCs) to elicit an immune response, often observed as a component of DILI. We then performed transcriptome evaluation (TempOseq) of the PBMCs and explored the pathways that were differentially activated between the two risk categories of compounds. The most significant dysregulated pathways within the most DILI concern include innate and immune response and apoptosis. In addition, we built a random forest machine learning model to evaluate the predictive value of transcriptomic *in vitro* screening. Even in the absence of final clinical dosing information, the model was able to identify a significant proportion of the compounds of most DILI concern compounds. We then compared this transcriptomic-only model to a model built using simple physical/chemical properties and found that the transcriptomic-only model does not add additional quantitative value to computational prediction of overall DILI risk. However, we suggest that transcriptomic response adds novel value for understanding mechanisms of DILI for certain classes of compounds, in particular immune mediated toxicity.

2024 Enhancing the US EPA Adverse Outcome Pathway Database (AOP-DB): Recent Updates and Semantic Integration

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There is a need for more efficient use of existing data to characterize human toxicological response data for environmental chemicals of interest to the US Environmental Protection Agency (EPA). The Adverse Outcome Pathway (AOP) framework helps to organize existing mechanistic information, where AOP data is currently submitted directly by users and stored in the AOPwiki. Automatic and systematic parsing of AOPwiki data is challenging, so we have created the EPA AOP-DB. The AOP-DB is an AOP profiler, developed to assist in biological and mechanistic characterization of AOP data and provide a broad, systems-level overview of the biological context of AOPs. Here we present recent updates to AOP-DB version 2, including 262 AOPs from the AOP-wiki xml, AOP-Tissue Network and Human Susceptibility modules, and a computationally-predicted AOP Builder. We also present updates to the GUI frontend with search and download capabilities. Lastly, we describe recent semantic mapping efforts for the AOP-DB, and how this process integrates AOP-DB data with other toxicologically relevant datasets. *This abstract does not reflect US EPA Policy.*

2025 Hepatic and Renal Concentrations of General Chemicals Estimated after Virtual Oral Administrations Extrapolated Using Simple Physiologically Based Pharmacokinetic Models and Rat Plasma Data

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Recent high-throughput *in vitro* screening assays combined with *in silico* computational models might provide suitable alternative methods to conventional animal testing. The aim of the present study was to model the plasma, hepatic, and renal pharmacokinetics of approximately 70 disparate types of chemicals and drugs after virtual oral administrations in rats based on reported rat plasma values and experimental pharmacokinetics determined after oral administration to rats for evaluating toxicological potential. To ensure that the substances analyzed exhibited a diversity of chemical structures, the structures defined by 196 chemical descriptors in a chemoinformatics tool were calculated for 50,000 randomly generated molecules in the original chemical space. To allow visualization, the resulting chemical space underwent projection onto a two-dimensional plane using generative topographic mapping. The current study employed a one-compartment model recently recommended by US authorities and a physiologically based pharmacokinetic (PBPK) model made up of chemical receptor (gut), metabolizing (liver), excreting (kidney), and/or central (main) compartments. The plasma concentration curves and the maximum concentrations of a varied selection of approximately 70 chemicals obtained by high-throughput one-compartment models and our simple PBPK models were consistent. However, the hepatic or renal and plasma concentrations or the areas under the concentration-time curves of 70 chemicals were different between the PBPK modeling and empirically obtained values. Although the numbers of compounds were limited, the lowest observed effect level values for hepatoxicity or nephrotoxicity of several compounds from the Hazard Evaluation Support System Integrated Platform in Japan and the areas under the hepatic or renal concentration-time curves estimated using PBPK modeling were inversely associated. The present models could estimate the relationships between plasma/tissue concentrations of chemicals and drugs after oral doses using both forward and reverse dosimetry with a view to predicting hepatic or nephrotoxic toxicity as a part of chemical risk assessment. *Supported by the METI Artificial Intelligence-based Substance Hazard Integrated Prediction System Project, Japan.*

2026 Gaining Confidence in Combined Methods: Uniting Structural Alerts, Random Forests, and Neural Networks for Use in Risk Assessment

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Molecular Initiating Events (MIEs) provide good targets for computational modelling, as they are well defined chemical-biological interactions. When compared to trying to predict biological endpoints, MIE predictions do not pass over large amounts of biological complexity. Computational approaches based on the chemistry of chemical binders have been developed to make predictions at pharmacologically important human MIEs, and these approaches have been combined to provide a high performing model. The first computational model uses structural alerts developed automatically using maximal common substructure searches and Bayesian statistics in KNIME. In addition to this, two different machine learning algorithms have been used - random forests and neural networks. The random forest models were constructed using sklearn in RDKit, with 200 physicochemical descriptors as the input. The neural networks were developed in Python 3 using TensorFlow. Extended connectivity fingerprints were used as inputs and a variety of network architectures considered to give the highest level of statistical performance. All three computational approaches consistently provide models with over 90% accuracy against test data. The data used for model construction was consistent across all three approaches, with ChEMBL and ToxCast data being combined to provide a relatively balanced dataset between experimental positives and negatives. Biological targets assessed include G-protein coupled receptors, nuclear receptors, enzymes, ion channels and transporters to give a balance of different human MIEs. While the developed computational models provide high levels of performance, combining them in a decision-making context allows us to use the advantages of each method to provide the best possible prediction. In addition, this procedure shows an increase in model performance. Applicability domains and confidence scores for test chemicals can be used to better understand how new chemicals compare to the data set used in model construction. Finally, these models have been assessed on truly external biological data to test their effectiveness. This is the ultimate goal of such predictive approaches as it gives the best impression of their usefulness outside the training and test data. While there is a reduction in model performance in this scenario, it helps us understand how models perform when novel chemicals are assessed, and hence how they are useful in risk assessments.

2027 *In Silico* Prediction and Suggestion to Alleviate Promiscuity of Compounds

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Since safety is one of the most influential factors of drug attrition in drug discovery, the selection of compounds with less toxicity concerns is important to increase the success rate of drug discovery. To estimate the concern of off-target toxicity during early research stages, we originally constructed a small-scale assay panel, promiscuity panel (PP) [Sameshima, T. et al. Chem. Res. Toxicol. 2019], consisting of eight targets (ROCK1, PDE4D2, GR, PPARγ, 5-HT_{2B}, Adenosine A_3, M1 and $GABA_A$) to evaluate binding properties towards a wide range of gene families. To promote the optimization of compounds more efficiently, cheminformatics analysis of large amount of assay results is expected to be useful. We applied two types of *in silico* approaches to assay results of PP. One is a quantitative structure-activity relationship (QSAR) modeling to aim prediction of binding property. In this study, we constructed QSAR models based on PP assay results of approximately 3000 compounds by using random forest method. Although the QSAR model of GR showed low predictability because of its low hit rate, the other seven QSAR models showed high predictability with more than 0.86 of AUC value of ROC curve by 5-fold cross validation. By using PP QSAR models, we evaluated compounds from Liver Toxicity Knowledge Base (LTKB) of FDA, and confirmed the relationship between a hit count of PP QSAR models and DILI severity. The other type of *in silico* approaches is a matched molecular pair (MMP) analysis to aim suggestion of structural modification. MMP is defined as a pair of molecules that differ only by a single localized structural change. By analyzing assay results of PP, several dozens to several hundreds of MMPs were successfully extracted in each target except GR. Among them, several dozens of substructure pairs contributed to alleviation of each target potency. Interestingly, some of them reduced multi-target potency simultaneously. Extracted knowledge about structural transformation would provide more useful suggestion for chemical design to reduce potency on targets in PP by simple transformations. Prediction by PP QSAR models and suggestion based on MMPs would provide useful information in optimizing compounds to alleviate binding promiscuity.

2028 Predictive Models for Human Organ Toxicity Based on *In Vitro* Bioactivity Data and Chemical Structure

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Traditional toxicity testing reliant on animal models is costly and low throughput, posing a significant challenge with the increasing numbers of chemicals that humans are exposed to in the environment. The purpose of this investigation was to build optimal prediction models for various human *in vivo*/organ level toxicity endpoints using chemical structure and *in vitro* quantitative high-throughput screening (qHTS) bioactivity assay data. Several supervised machine learning algorithms were applied to model 14 human toxicity endpoints such as vascular, kidney ureter and bladder and liver. Three metrics were used to evaluate model performance: area under the receiver operating characteristic curve (AUC-ROC), balanced accuracy (BA), and Matthews Correlation Coefficient (MCC). Overall, the three metrics showed the same trend for each endpoint. Models for the three endpoints achieved good performance with AUC-ROC values >0.8, including musculoskeletal (0.84±0.02), endocrine (0.82±0.01), and peripheral nerve and sensation (0.81±0.01) toxicities. In addition, the model performance was found to be dependent on the specific dataset and model type. Nonetheless, *in vitro* assay data combined with chemical structure improved the predictive accuracy for most endpoints (8 out of 14). Chemical structure and assay data showed different levels of contribution to the prediction of different toxicity endpoints. The top performing models from this study can be further applied as a tool for hazard assessment to screen large sets of chemicals for potential human toxicity. In addition, the models identified structure features and cellular targets that contributed the most to each toxicity endpoint, which could serve as a guide for chemical prioritization and better understanding of toxicity mechanisms.

2029 Exploring IVIVE for Exposure and Health Impacts of E-Cigarette Flavor Mixtures

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In vitro to *in vivo* extrapolation (IVIVE) refers the processes that leverage *in vitro* experimental results to predict corresponding *in vivo* measurements. This approach can be used to estimate clinical exposure scenarios that may pose an adverse health risk based *in vitro* responses, potentially bypassing the need for animal testing. Interpretation of IVIVE results and confidence in modeling predictions are affected by model type, exposure route and kinetic assumptions for the test article, and choice of *in vitro* assay(s). Exposure scenarios can be complicated, particularly for mixtures. For example, clearance rates for individual chemicals are obtainable experimentally or via QSAR models but generating comparable rates for mixtures remains an open research area. The *in vitro* assay selected and its relevance to the *in vivo* endpoint of interest are also critical in interpreting biological relevance of prediction results. Choosing an assay unrelated to the mode of action for human toxicity endpoint will lead to misrepresentation of *in vivo* effects. Here we use e-cigarette (EC) aerosols, a complex mixture including carriers, flavors and nicotine, as a case study to explore IVIVE modeling approach. We utilized literature *in vitro* cytotoxicity data on EC flavor mixtures to predict exposure scenarios that could lead to adverse toxicities. Several pharmacokinetic models were explored including a simple steady state model and a 3-compartment model with repeat dosing and multiple exposure routes. Additionally, impacts of *in vitro* assay endpoints on e-liquid consumption (number of cartridge or pod) estimates were evaluated using publicly available mechanistic (Tox21) *in vitro* data for individual flavors. Our results suggest that depending on the modeling approach and *in vitro* assay selection, the e-liquid consumption predicted to obtain corresponding *in vivo* plasma levels can be unrealistically high. For example, >1,000 pods (>700 mL e-liquid)/day was estimated for human exposures for certain flavors using standard cytotoxicity measures. In contrast, mechanistic (Tox21) assays yield a lower but wider exposure range (3 to 100,000 pods) for some flavors. These proof-of-concept results highlight challenges and complexities in IVIVE for mixtures. Limitations and considerations for assay selection and an example decision tree to facilitate use of IVIVE in projecting exposure and health impacts of complex test materials such as flavor mixtures is provided.

2030 Using SEND Datasets to Enable Large-Scale Data Analytic Approaches for Preclinical Toxicology Data

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BioCelerate, a subsidiary of TransCelerate BioPharma, Inc., is a preclinical industry consortium driving initiatives to increase efficiency and productivity in early stage R&D. The Standard for Exchange of Nonclinical Data (SEND) is a standardized format for capturing a wide range of toxicology-study data. Since 2016 the US FDA has required sponsors to submit data from certain types of toxicology studies in SEND format. The implementation of the SEND model represents a tremendous opportunity to apply large-scale data analytic approaches to preclinical toxicology data. Before this opportunity can be realized, differences in SEND implementation that make it difficult to conduct cross-study analysis must be addressed. The BioCelerate consortium has been engaged in an effort to better enable these analytic approaches and identify solutions since 2016 and more recently has been in collaboration with the US FDA. The collaboration has identified areas in SEND data sets that are significant drivers of variability that negatively impacts cross-study analysis. Currently, BioCelerate and the FDA are working to propose an approach to address some of these issues that prevent more efficient cross-study analysis, and we have created six use cases for this evaluation. In this presentation we will focus on our background-control data use case and present a potential approach for improved data harmonization. Generating and analyzing background-control data can play a significant role in nonclinical development by defining the frequency of incidental findings across a range of specified conditions that can be used to interpret low incidence findings in a study of interest. For each parameter of interest in the background control use case, we present how to extract this information from SEND data and transform it if needed for analysis and a proposed methodology for how to structure database queries. While the current presentation will focus on background-control data, these approaches can be used for all six of our identified use cases. By implementing our proposed approaches, stakeholders will be able to take advantage of the opportunity presented by SEND datasets to enable increased efficiency and productivity in early stage R & D.

2031 Development of a Computational Profiler for Inhibition of Mammalian Steroidogenesis

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The conversion of cholesterol to steroid hormones is a key process in physiology and development. Disruption of the steroidogenesis pathway is associated with developmental disorders and as such, it is prudent to identify chemical disruptors of steroidogenesis within the early stages of the product development cycle. To this end, we compiled a database of chemicals that inhibit the enzymes which catalyze key steps in the synthesis pathway for estrogens and androgens (P450scc, CYP17A1, 3β-HSD, 17β-HSD, aromatase and 5α-reductase). These data were curated from public data sources and peer-reviewed literature. Using the open-source KNIME platform, we developed computational models to identify conserved structural motifs (scaffolds) associated with organic compounds that inhibit each of these enzymes. The resulting profiler output was combined into a pan-steroidogenesis profiler wherein a chemical that contains a scaffold known to inhibit any of the assessed enzymes is flagged as a potential steroidogenesis disruptor. To validate the model, we tested its ability to identify compounds that decrease estradiol production in the ToxCast high-throughput H295R assay. Our model was able to identify chemicals flagged as inducing a reduction in cellular estradiol production with 75% sensitivity, 83.3% specificity and 79.1% balanced accuracy. Given the heterogeneity of targets associated with the modeled endpoint and the fact that our profiler involves identification of conserved structural scaffolds, these concordance statistics are considered sufficiently accurate. We further validated our model against a second dataset of 31 compounds from the NTP integrated chemical environment (ICE) dashboard that were assessed for their ability to inhibit estrogen biosynthesis *in vitro*. Of the 20 chemicals that decreased estrogen biosynthesis, our model flagged 14 compounds, yielding 70% sensitivity (with 81.8% specificity and 75.9% balanced accuracy). Assessment of the remaining 6 chemicals suggest they are likely false-positives from the *in vitro* assay since they represent potential mitochondrial toxicants that inhibit oxidative phosphorylation or agonists of nuclear receptors (AhR and androgen receptor). In conclusion, these findings demonstrate the utility of our profiler in identifying potential steroidogenesis inhibitors as a first-tier screen for new chemicals.

2032 Using *In Silico-In Vitro* to *In Vivo* Extrapolation to Predict the Oral Dose Required to Activate the Aryl Hydrocarbon Receptor (AhR)

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Activation of AhR can have toxic effects on mammalian hosts. We used *in silico* and *in vitro* to *in vivo* extrapolation to automate the prediction of the dose required to activate AhR in humans. About 1,000 compounds were identified as human AhR activators (AC50 < 100 uM) by a Tox21 assay. We extracted human hepatocyte intrinsic clearance (CLint) and human fraction unbound to plasma (Fup) experimental data from the httk R Package[1] to use as *in vitro* inputs in the simulations. All other parameters, e.g., solubility, logP, pKa, used were estimated with *in silico* models. The final data set was comprised of 160 compounds. The dose required to reach a steady state unbound plasma concentration equal to each compound's AhR AC50 concentration was predicted using the HTPK Simulation Module in ADMET Predictor® 9.5. If the AhR AC50 value isn't reached with a 1,000 mg dose, then the run is terminated. The percent fraction absorbed (%Fa) and oral bioavailability was then predicted using the estimated dose. The AhR AC50 values in the data set ranged from 0.043 to 74.1 µM. *In vitro* Fup values ranged from 0.0 - 1.0, with 38 compounds having values less than or equal to 0.005. Fup values below 0.005 were set to 0.005 in the simulations. CLint values ranged from 0 to 1,000 µl/min/million cells, with 35 of the compounds having CLint=0 µl/min/million cells. A large majority of the compounds (142) were unable to reach an unbound steady state concentration equal to their AhR AC50 value with up to a 1g dose. 30 of these compounds were predicted to have low %Fa (<= 30%), mostly due to low solubility. 18 compounds were predicted to be potential *in vivo* AhR activators with a dose below 1 g. The minimum, maximum, average, and median predicted dose are 13, 912, 438, and 451 mg, respectively, for these compounds. Among these, isoniazid, an antibiotic used for the treatment of tuberculosis, was predicted to have the lowest dose to reach its AhR AC50 value (0.573 µM). Isoniazid's drug label contains a warning for hepatitis and an acetaminophen drug-drug interaction. It has been proposed that isoniazid increases the amount of acetaminophen converted to its toxic metabolite since activation of AhR induces cytochrome P450 expression[2]. [1]Pearce RG,

Setzer RW, Strope CL, Sipes NS, and Wambaugh JF. httk: R Package for High-Throughput Toxicokinetics, *J Stat. Software*, 2017, 79, 4. [2]Larigot L, Juricek L, Dairou J, and Coumoul X. AhR signaling pathways and regulatory functions, *Biochimie Open 7*, 2018, 1-9.

2033 ComptoxAI: A Tool Kit for AI Research in Computational Toxicology

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Cutting-edge data science and informatics techniques stand to revolutionize computational applications in toxicology, but data and techniques for interacting with toxicological phenomena are fragmentary, sparse, and poorly understood. With this in mind, we present ComptoxAI - an artificial intelligence toolkit for toxicology that integrates public data from diverse sources and enables a wide array of advanced computational analyses. ComptoxAI includes a large graph database of toxicology-related concepts, a Python library for interacting with the data, a collection of graph algorithms and machine learning models for making new discoveries, and a complete ontology to formally define entities in toxicology research and the complex relationships between them. At the present time, the graph database and ontology contain structured representations for 72,921 entities and 1,848,307 relationships between those entities. Development of ComptoxAI is ongoing and highly active, and we have already used it to make several new discoveries related to toxicology data. For example, we computed the most influential nodes in ComptoxAI's graph database using the PageRank algorithm, and found that when Adverse Outcome Pathway (AOP) data are included in the analysis, the list of the most influential genes in the network is dominated by those involved in metabolizing and clearing toxicants from the human body (such as *abcb1* and various Cytochrome P450 genes). When AOP data are excluded from the analysis, this effect disappears almost entirely, which suggests that AOPs effectively encode important information about mechanisms of toxicity, and that ComptoxAI can easily detect such patterns using graph theory alone. The planned future work on ComptoxAI includes the inclusion of deep learning models to discover new associations between toxic exposures and downstream adverse effects, and creating improved interfaces for querying and interacting with toxicology data (particularly for researchers without computer programming expertise), among other research projects. ComptoxAI is open-source, and can be found online at https://comptox.ai. *Supported by grants P30-E013508 and T32-ES019851.*

2034 Evaluation of Existing QSAR Models and Structural Alerts and Development of New Consensus Models for Genotoxicity Using a Newly Compiled Experimental Dataset

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Genotoxicity is among the toxicological endpoints that pose the highest concern for human health and is subject to regulatory assessment. Integrated testing and assessment approaches (IATA) are applied to evaluate the genotoxic potential of chemicals using a combination of *in silico*, *in vitro* and *in vivo* approaches. A major effort was undertaken to compile data from several sources (TOXNET, COSMOS, eChemPortal and ECVAM) and harmonize the names and outcomes of different assay types (bacterial mutagenicity (Ames), chromosomal aberrations (Clastogen), and others). The data was evaluated using a conservative IATA to assess genotoxic potential using the classification scheme by Williams et al., 2019. The dataset comprised 4828 chemicals, of which 2553 chemicals were categorized as genotoxic and 1819 as non-genotoxic using the IATA. The IATA assigned a chemical as genotoxic if any single Ames or Clastogen assay was positive; ~16% chemicals active in <50% Ames assays were classified as genotoxic and ~15% chemicals with inconclusive Ames data were classified as genotoxic. So, a new cut-off-based classification scheme was developed where genotoxicity classifications were made based on percentage of Ames and Clastogen assays a chemical was active in. Next, QSAR tools (TEST and Lazar) and the OECD Toolbox structural alerts/profilers (e.g. OASIS DNA alerts for Ames, CA) were used to make *in silico* predictions for genotoxicity. The performance of individual QSAR tools and alerts was evaluated against the IATA and newly defined cut-off-based genotoxicity classifications. The balanced accuracies ranged from 64-80%. Finally, a naïve Bayes consensus model was developed using two combinations of QSAR tools and alert predictions. The consensus models do not result in significant differences in the overall prediction across various combinations, with balanced accuracies ranging from 50-76%. Overall, the predictivity of individual tools and the consensus models is slightly improved using the experimental activity cut-off-based classification scheme relative to the Williams et al., 2019 scheme for genotoxicity prediction. These models will provide a robust support framework for assessing genotoxicity potential for new and untested chemicals. *This abstract does not necessarily represent US EPA policy.*

2035 A Pipeline for Prediction of Developmental Toxicants: Screening in Zebrafish and Correlation to HTS Data

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The zebrafish embryo is an effective alternative *in vivo* model to rodents for studying chemically-induced perturbations because of its small size and rapid development. In addition, the availability of transgenic embryos that express fluorescence in specific tissues or organs allows for detection of tissue-specific structural malformations (apical end-points). We performed screening for skeletal and angiogenic disruption in transgenic zebrafish embryos caused by exposure to Phase I chemicals from EPA's Toxicity Forecaster (ToxCast) program. Around 300 chemicals were screened and we identified 38 skeletal and 10 angiogenic disruptors. The identified sets of skeletal and angiogenic disruptors were used to identify ToxCast assays that were affected by the same chemicals using univariate correlation analysis. Identified assays included those that measure vitamin D_3 metabolism and dopamine transporter activity, and oxidative stress and nuclear receptor signaling for skeletal and angiogenic disruption, respectively. We further used the identified assays to make predictions of other chemicals, tested in the Tox21 screening project, but not tested for skeletal and angiogenic disruption in zebrafish embryos, using the Toxicological Priority Index (ToxPi) program. This program ranks compounds based on their AC50 value for the selected assays. Predicted disruptors were tested in the laboratory, and several new skeletal and angiogenic disruptors were identified. We conclude that medium throughput screening data from an *in vivo* model in combination with modeling of ToxCast high throughput *in vitro* data can be used to produce testable hypotheses on modes of action of chemical exposures, and to predict apical end-point specific effects of chemical exposures. *Disclaimer: This abstract does not necessarily represent US EPA policy.*

2036 Harnessing *In Silico*, *In Vitro*, and *In Vivo* Data to Understand the Polycyclic Aromatic Compound (PAC) Landscape

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Polycyclic aromatic compounds (PACs), including polycyclic aromatic hydrocarbons (PAHs), are ubiquitous environmental contaminants that can arise from incomplete combustion or thermal decomposition of organic matter. While many of the PACs are linked with carcinogenicity and some are linked with reproductive/developmental toxicity, still many members of the class do not have toxicity data available. Thus, there is a need to leverage the available data (e.g., *in silico*, *in vitro*, and *in vivo*) for rapid hazard characterization. To this end, we created a computational workflow to automatically identify PACs in a library (e.g., Tox21, ~ 9K compounds). Then, we clustered the PACs based on their *in silico* toxicity profiles including 8 different categories (carcinogenicity, cardiotoxicity, developmental toxicity, genotoxicity, hepatoxicity, neurotoxicity, reproductive toxicity, and urinary toxicity). We found that PACs with the same parent structure (e.g., fluorene) were predicted to have diverse toxicity profiles. Moreover, PACs with similar substituted groups (e.g., alkylated-PAHs) or heterocycles with varying ring sizes (e.g., N-PACs) were predicted to have similar toxicity profiles. By surveying the *in silico* toxicity and *in vitro* activity profiles in Tox21 assays, we found that toxicity endpoints related to genotoxicity/carcinogenicity and effects related to xenobiotic homeostasis (aryl hydrocarbon receptor -AhR, constitutive androstane receptor -CAR) and stress response (p53 and Nrf2 pathways), respectively drive the activity variation seen in the PACs. The availability of *in vivo* data (e.g., carcinogenicity) in conjunction with toxicity/activity profiles were inspected to identify 'hot spots' - either data-poor (hydroxylated PAHs) or data-rich (unsubstituted, parent PAHs) regions. These regions are targets for read-across evaluation of the unknowns, and potentially, *in vivo* assessment. In summary, we have developed a strategy to explore the PAC landscape utilizing *in silico*, *in vitro*, and *in vivo* data. This strategy can be extended to other types of compounds which need hazard evaluation.

2037 Development of a Computational Prediction Model for Modulators of the Androgen Receptor (AR)

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Endocrine signaling via the androgen receptor (AR) plays a crucial role in mammalian physiology, development and pathology. As an initial step towards modeling the key events within adverse outcome pathways (AOPs) for AR-mediated endocrine disruption, we developed an *in silico* model to predict the likelihood that an unknown compound will or will not bind the AR. We mined existing public data and compiled a database of >2400 AR binders, together with >2100 controls (non-binders) from multiple sources (ToxCast, CHEMBL, published literature, etc.). We developed a random-forest machine-learning model using structural fingerprints and the presence or absence of 2D-structural motifs (scaffolds) conserved within the known AR binders as covariates. Our prediction model was built by iteratively and randomly retraining it using 90% of the compiled active and inactive (control) compounds, with the remaining compounds serving as the test set. The resulting model shows very high sensitivity (92.8%), specificity (94.6%) and balanced accuracy (93.7%). This is coupled with high positive (95.2%) and negative (91.9%) predictive values, highlighting the confidence with which assessed substances can be aligned as AR binders or non-binders. By iteratively building our model using 1-90% of the actives (AR binders), we show that <39% of the curated AR binders are needed to yield >90% sensitivity, highlighting the robustness of the model and suggesting that it will not change for the foreseeable future. In conclusion, these findings demonstrate the feasibility and utility of using public *in vitro* data to develop a computational model to identify potential AR binders as a first-tier *in silico* endocrine disruption screen for new chemicals.

2038 Using Gene Expression Data to Identify Liver and Kidney Injuries

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The immense resources required and ethical concerns for animal-based toxicological studies have driven the development of *in vitro* and *in silico* approaches. Using gene expression data from liver and kidney tissues of rats exposed to diverse chemical insults, we previously derived a set of gene modules associated with specific organ injuries (i.e., injury modules). Recently, we validated this approach in a study using thioacetamide, a known liver toxicant that promotes fibrosis. Our first aim was to test whether we could use gene expression from rat primary liver and kidney cells (*in vitro*) exposed to thioacetamide to predict organ injuries in rats *in vivo*. Second, we sought to establish interspecies concordance between the gene module responses in rat and human primary cells exposed to thioacetamide to predict organ injuries. In all cases, the most activated liver gene modules were those associated with fibrosis. Histological analyses supported these results, demonstrating the potential of gene expression data to identify organ injuries. The *in vitro* predictions were significantly correlated with the *in vivo* predictions, with an R^2 value of 0.64. Finally, the top-ranked liver injuries in human primary cells correctly identified known pathological changes such as fibrosis. Our proposed approach could potentially be used with *in vitro* assays to screen large number of chemicals and predict liver and kidney injuries *in vivo*.

2039 Use of Computational Toxicology (CompTox) Tools to Predict *In Vivo* Toxicity for Risk Assessment

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Open access CompTox tools were examined for use in risk assessment by comparing *in vitro* predictions to *in vivo* pesticide toxicity. The pesticides, propyzamide (PZ: amide), carbaryl (CB: carbamate) and chlorpyrifos (CPF: organophosphate), were chosen because of their well-characterized toxicological profiles. The CompTox tools included: 1) CompTox Chemistry Dashboard *in vitro* bioassays (AC_{50} μM: activity at 50% concentration) with values below the cytotoxicity limit for each chemical (region where activity may not be specific); with assay components relevant to the toxicity pathways and with no disqualifying flags; 2) The selected CompTox AC_{50}s were converted by *in vitro*-to-*in vivo* extrapolation (IVIVE) to produce oral equivalent doses (OED: mg/kg/d; steady state plasma concentration Css); and 3) the CompTox AC_{50}s were entered in the high throughput toxicokinetics (HTTK) R program to get age-adjusted equivalent doses (mg/kg/d; Css). Data conversions by IVIVE or HTTK were compared to known *in vivo* lowest effect levels (LEL÷10x interspecies extrapolation) from repeat dose studies as follows: CB and CPF LELs (0.1 mg/kg/d) from preweaning rat studies had P450 (CYP) induction and brain acetylcholinesterase (AChE) inhibition as a mode of action (MOA). PZ LEL (1.0 mg/kg/d) from a pubertal rat study had decreased T_4 after CYP and glucuronosyl-transferase (UGT) induction as a MOA. AC_{50}s for assays with components known to be associated with these MOAs (e.g. PZ: CYP1A2, UGT1A1, CB: CYP1A1/2, AChE, AhR; CPF: PXRE, PXR) produced IVIVE results with OED:LEL ratios of 9.8 - 38 (PZ), 8 - 69 (CB) and 3.4 - 4.17 (CPF) indicating that each chemical had a ratio within an order of magnitude of their respective *in vivo* LELs. HTTK produced *in vivo*-equivalent doses for children 6-11 and 11-15 coinciding with the preweaning and pubertal ages of the rats in studies determining the *in vivo* LELs. HTTK age-adjusted equivalent dose-to-LEL ratios for PZ and CPF were 2.3 - 9.2 and 1.43 - 2.09, respectively (predictive association), where CB had a much higher ratio (44 – 373, less predictive ratios). Overall the tools had value for predicting equivalent doses that were close to the known *in vivo* LELs for the relevant metabolic pathways. However, data interpretation was limited by having few *in vitro* assays below the cytotoxicity limit that were key to the toxic pathways (e.g. AChE for CPF) which may have been due to a lack of metabolic activation. Nevertheless, these tools may facilitate mechanistic insights or selection of a point of departure in risk assessment for chemicals with unknown toxicity.

2040 An *In Vitro-In Silico* Model for Characterizing Hazard and Population Variability in Cardiotoxicity Induced by Environmental Chemicals

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Population variability in toxicodynamic (TD) susceptibility remains a critical data gap in toxicology, with human health risk assessments largely relying on a default $10^{1/2}$-fold factor. This creates a need to establish a high-throughput, physiologically relevant, and accurate framework to address this data gap. We tested the hypothesis that combining a population-based human induced pluripotent stem cell derived (HiPSC) cardiomyocyte model and Bayesian Markov Chain Monte Carlo modeling could be used to characterize cardiotoxicity hazard and quantify population TD variability in a chemical- and endpoint-specific manner. Here we screened 138 different compounds, representing pharmaceuticals, environmental chemicals, industrial chemicals, and food additives, in cardiomyocytes derived from 43 distinct individuals. TD variability is calculated for cell viability as well as for functional parameters related to beat rate, prolonged action potential, and irregular beating. For each of the 138 chemicals, Bayesian population concentration response modeling was employed to estimate points of departure for each endpoint. For compounds that caused significant population level responses in the tested concentration range, a toxicodynamic variability factor (TDVF) was also derived, which can be used as a chemical-specific adjustment factor to replace the default $10^{1/2}$ uncertainty factor. Overall, TDVF values tended to be larger than the default TD uncertainty factor and the functional endpoints were more variable than the viability endpoint. Despite the increased population variability however, an IVIVE analysis shows that there is a large margin of exposure between most environmental compounds and functional endpoint PODs. In this study, we demonstrate how our model can be used as an accurate, animal-free, human-relevant, and tissue-specific predictive tool for addressing the challenge of incorporating population TD variability in human health risk assessments.

2041 Identifying Individual and Co-occurring Chemicals That Induce Changes in Transcription of Cancer and Inflammation-Related Genes upon *In Vitro* Exposure to Ambient Air Pollution in Houston, Texas

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Atmospheric pollution represents a complex mixture of air substances that continually interact and transform, making it difficult to accurately evaluate toxicity responses attributable to individual and/or co-occurring chemicals. *In vitro* studies that evaluate air pollution mixture toxicity have largely been

reliant upon laboratory-generated exposure scenarios that do not reflect real-world conditions, heavily weighted towards individual chemical-based assessments. To address this gap, this study set out to evaluate lung cell toxicity directly induced by outdoor atmospheric pollution using field-based exposure conditions in the industrialized Houston Ship Channel. The tested hypothesis was that individual and co-occurring chemicals in the atmosphere induce transcriptional changes of critical genes involved in cancer and inflammation signaling. Human lung cells were exposed at an air-liquid interface to ambient air mixtures using the novel Gas Phase *In Vitro* Exposure System (GIVES) for four hours, with experiments replicated across five days. Real-time monitoring of 31 primary and secondary gas-phase pollutants, as well as other atmospheric conditions, was simultaneously carried out. Co-occurring chemicals were identified and aggregated using weighted co-expression statistics. Transcriptional analysis of the exposed cells identified critical genes showing differential expression associated with individual chemicals, as well as aggregated measures of co-occurring chemicals. The individual pollutant identified with the largest associated transcriptional response was benzene, identified to associate with the increased expression of genes relevant to the inflammasome complex formation, including the NLR family pyrin domain containing 3 (*NLRP3*). Analysis of transcriptional response to co-occurring chemicals have identified overlapping responses, though also indicate a subset of discrete modulations involving separate gene sets. This study is among the first to measure lung cell transcriptional responses to both individual and co-occurring chemicals in relation to real-world, ambient air mixtures.

PS 2042 Expanding Access to Safer Chemistry for Consumer Products throughout the Supply Chain: Tools and Trends

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Historically consumer product safety has focused on regulatory compliance through Safety Data Sheet disclosure, Authoritative List Checking (ALC), and analytical testing. Leading consumer products brands (LS&Co, Nike, H&M, among others) are driving safer chemistry selection throughout the supply chain, moving beyond compliance by evaluating human and environmental health characteristics. The authors present recent work on tools in use by chemical suppliers, product formulators, textile mills, brands, and retailers, transforming the way chemical selection is occurring for consumer products. The chemical selection process is facilitated by an innovative combination of Qualitative and Quantitative Comprehensive Hazard Assessment (QQCHA) methods, safer alternative selection via functional use groups (FUGs), chemical and formulation scoring, and integrative methods for downstream knowledge, all of which are aided by online software. The textile industry is at the forefront of the movement towards upstream safer chemical screening, assessment, scoring, and selection. One highly successful program is Screened Chemistry (SC). SC requires Confidential Full Formulation Disclosure (CFFD) in textile formulations. The authors have screened over 350 formulations with hundreds of chemicals under this program. The results show progress throughout the supply chain in achieving CFFD and selecting safer chemistries. For CFFD, information exchange via software allows formulators to quickly share SC scores with redacted ingredient data, maintaining confidential business information. Instant availability of QQCHA results for over 2,000 commonly-used chemistries dramatically reduces the cost of safer chemistry selection in consumer products with thousands of additional instant results for unacceptable or potentially safer alternatives available for formulators to explore. The authors will discuss the QQCHA methods, the critical deficiencies of list-based "hazard screening", trends in scored chemistries used in textile formulations to date. The results show how the tools and use of software have effectively made comparative evaluation of potential safer alternatives more robust than traditional methods such as ALC or testing used alone or combined. Looking forward the authors will discuss integrative methods to inform brands, retailers, and consumers of safer chemistry achievement for products and processes.

PS 2043 Developmental Computation with Embryonic Stem Cells

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New approach methodologies (NAMs) that enable *in vitro* profiling of chemical-biological interactions come with the need for *in silico* models that translate vast amounts of data and information into toxicological prediction. For fetal systems, these models must reflect the best available knowledge of embryology. A new ToxCast platform using pluripotent embryonic stem cells (H9 line) identified a signal for developmental toxicity potential in 183 of 1065 chemicals (17%) [Zurlinden et al., submitted]. Recursive partitioning using 5-fold cross validation on 80/20 split produced binary classifier models for ToxRefDB developmental toxicity with balanced accuracies of 65% (431 chemicals) to 88% (127 chemicals). PI3K-FOXO signaling was hypothesized as a major determinant of sensitivity. RNAseq profiles for retinoic acid-induced effects on endoderm-directed stem cells (eg, FOXA2, SOX17, TBXT, EOMES, LHX1, BMP4) revealed a tipping at 17 nM [Saili et al., 2019]. Here, we expand the performance-based models by a deep-learning strategy that brings embryology of these pathways into the fold using a Compucell3D agent-based model (ES-ABM) to unravel chemical effects on signals/responses for self-renewal (unlimited proliferation), pluripotency (broad differentiation potential), and self-organization (rudimentary anatomical structures). ES-ABM is initially designed to model stochastic dynamics of locally interacting stem cell agents during retinoid concentration x time exposure, conveying mechanistic hypotheses about key events for chemical-induced alterations of stem cell differentiation and self-organization. *This abstract does not reflect US EPA policy.*

PS 2044 Gene Co-regulated Network Analysis Applied to Big Data in Toxicology: TXG-MAPr, a Flexible Tool to Integrate and Understand Transcriptomic Data

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What are the gene expression patterns underpinning adverse outcome occurrences *in vivo*? How can we measure sensitivity of primary cell donors towards a toxicant by deconvoluting their transcriptomic profiles? These and similar questions can be tackled by applying gene network approaches to large toxicogenomic datasets. Herein, we present weighted gene co-expression network analysis (WGCNA) based on TG-GATEs datasets, including primary human hepatocytes (PHH), rat *in vivo* liver and rat *in vivo* kidney, housed in a web-Shiny App, the TXG-MAPr. The TXG-MAPr presents functional clusters of co-expressed genes (modules) that bridge between individual gene variations and emergent global properties, capturing dynamics of experimental conditions (compounds, doses and time). Modules are annotated for biological function and transcription factor regulation. Newly generated transcriptomic data can be analyzed in the context of the established gene networks in both cellular and *in vivo* systems. We show that PHH-derived modules can discern individual primary hepatocyte donor variability upon exposure to the same drug. Also, we show connectivity between activation of rat liver modules with the clinical chemistry and histopathology variations, thus identifying gene expression patterns concurrent and predictive of organ-specific apical endpoints. Moreover, selected module responses can serve as key events for Adverse Outcome Pathway construction. In conclusion, the TXG-MAPr represents a flexible and innovative tool contributing to the mechanistic understanding of potential adverse drug reactions. *The work received funding from the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking for the TransQST (agreement 116030) and eTRANSAFE (agreement 777365) projects, supported by the European Union's Horizon 2020 research and innovation program and EFPIA. This work was also supported by the EU-ToxRisk project, funded by the European Union's Horizon 2020 research and innovation program (agreement 681002).*

PS 2045 Use of a Computational Model for Transient Receptor Potential Vanilloid Subfamily Type 1 Protein (TRPV1) to Profile Sensory Irritants

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We are developing computational models that predict interactions with important biological targets, including those that could be informative for setting inhalation exposure guidelines. Sensory irritation of the nose, throat and eyes is frequently used as a critical effect in setting occupational exposure limits (OELs). Sensory irritation potential is determined by inhalation exposure of rodents to different concentrations of test material to derive the concentration that causes a 50 percent reduction in respiration rate (RD_{50}). Threshold limit values (TLVs) often fall within 0.01 - 0.1 RD_{50}. We developed a computational model to predict interaction with one important target for sensory irritation: the transient receptor potential vanilloid subfamily type 1 protein (TRPV1; capsaicin receptor). Using public sources, we compiled a database

of compounds known to interact or not interact with TRPV1 and developed a random-forest machine-learning model that analyzes their structural fingerprints and conserved scaffolds while also incorporating binding energies and topologies from protein-ligand docking. The model is sensitive (96%) and accurate (83%), with a specificity of 70%. TRPV1 compounds had significantly higher binding energies vs. controls. When we queried a database of RD_{50} values for 205 compounds, our model preliminarily identified 130 that potentially involve interaction with TRPV1. Follow-up analysis is planned including examination of subcategories of the scaffolds as well as interaction via additional biological targets that affect sensory irritation.

2046 Computational Association of Talc Exposure and Ovarian Cancer

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Talc is a fibrous magnesium silicate used in industrial and consumer products as a lubricant, thickener, or moisture-absorbing agent. Toxic impurities include asbestos, other silicates, and heavy metals. This study investigates a potential association of talc usage and ovarian cancer where it is hypothesized that interactions between talc and endogenous estrogen contribute to carcinogenesis. To investigate this, online databases, docking software, predictive molecular modeling software, and literature were used to study whether the contaminants in talc induce similar interactions as asbestos in the human body. From this, a potential adverse outcome pathway (AOP) with three primary carcinogenic pathways was generated: upregulation of the estrogen receptor (ER), activation of the epidermal growth factor receptor (EGFR), and generation of reactive oxygen species (ROS). Talc was hypothesized to aggregate endogenous estrogen to the ER via cations along its long and spindle-like structure. Activation of ER induces the Ras pathway for downstream anti-apoptotic effects via c-Jun and c-Myc. Second, phagocytosis of talc molecules was hypothesized to stimulate presentation of matrix metalloproteinases (MMPs) on macrophage and neutrophil surfaces via interactions with the metallic components and contaminants of talc, promoting migration and proliferation of keratinocytes and activation of the EGFR. In addition, the similar structures of talc and asbestos is hypothesized to allow talc to dimerize and activate EGFR in a similar fashion. Activation of EGFR induces unregulated anti-apoptosis, cell proliferation, DNA transcription, metabolism, and expression of oncogenes. Lastly, phagocytosis of talc exposes the macrophages and neutrophils to its heavy metal contaminants that are hypothesized to trigger inflammasome activation and upregulation of HMGB1 and NaIp3 pathways, resulting in the release of inflammatory cytokines such as IL-1B and TNF-a. Furthermore, upon necrosis, NADPH oxidase generates ROS species that upregulate growth factors and inflammatory pathways and disrupt iron homeostasis, causing further DNA damage.

2047 Toxicity Prediction of the In-Commerce List Using High-Throughput Screening and Pathway Analysis

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New approach methodologies have reshaped the way to evaluate health risks from chemicals. Research efforts are finding new ways to identify potential toxic hazards from laboratory tests or even broader screening tests. This case study shows the use of computer modeling to convert non-animal toxicity data into human exposure levels that can be applied in a public health context. Experiments to predict body burdens of substances, also known as *in vitro* toxicokinetics, are used with computer models to convert experimental toxicity data into biologically relevant doses. New computer generated toxicokinetic data have been released from the latest release of the HTTK R-Software package. Bioactivity profiles of In-commerce List chemicals were converted into equivalent biological exposure levels in human and daily consumer product exposure. Dream-TK is a computer application designed to derive equivalent human doses from high throughput toxicity screening in the US EPA ToxCast™ database. With the aid of new predicted *in silico* and *in vitro* toxicokinetic data, Dream-TK was able to estimate daily exposure ranges and biologically potential hazard levels for various chemicals from Health Canada In-Commerce List. The analysis identified 43 of 80 chemicals that displayed substantial amounts of bioactivity across a wide range of biological endpoints and pathways. 22 of the most potent chemicals exhibited sub-micromolar bioactivity. 20 chemicals were identified as having multiple genotoxicity flags, across both the *in vitro* ToxCast™ and *in silico* QSAR results. In addition to positive endocrine activity predictions for 7 steroidal chemicals, 5 non-steroidal chemicals also had significant endocrine disrupting potential. Computer simulations suggests that 41 of 43 equivalent biologically exposure doses are several orders higher then estimated daily consumer exposure of these chemicals. The research work is supporting evidence for new tools and technology advancing risk assessments at Health Canada.

2048 Image-Based Assessment of *In Vitro* Toxicity Using Deep Learning

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In vitro toxicity testing is an important, time and cost-effective way of assessing the drug and compound toxicity profiles and investigation of toxicant mechanism of action. However, current *in vitro* methodologies are limited in scope due to a restricted ability to extrapolate bioeffects/MOA from previously studied compounds. To address this limitation, we explored the potential of using deep learning techniques to assess *in vitro* toxicity from phase contrast microscopy images of human B-lymphocytes, along with corresponding images fluorescently labeled for nucleus and cell-viability, in attempt to perform comparative analysis of cellular phenotypes from previously-studied chemical toxicants. Deep convolutional neural networks have shown state-of-the-art performance in various image recognition tasks due to their ability to detect and learn highly nonlinear and complex image-based representation or features that can be used for image classification tasks. Specifically, we explored the following related hypotheses: (i) non-lethal exposure of human lymphocytes to a compound causes gross morphological changes in cells that are captured in the microscopy images with sub-micron resolution; (ii) the morphological changes are dependent on dose and the mechanism of action of a compound; and, finally (iii) convolutional neural networks have the potential to effectively use the information about the aforementioned morphological cellular changes as image features to learn the differences between cell exposure either or both to different doses of a compound or to different compounds having different modes of action. A preliminary convolutional neural network model trained on the image data was successfully able to distinguish between different doses, however, unsurprisingly, similar doses were less distinguishable. Likewise, some but not all compounds tested showed different morphological changes unique to each compound. This pilot study demonstrates the strengths and limitations of using microscopy images with convolutional neural networks for assessing toxicity in an *in vitro* system.

2049 Predicting Developmental Toxicity Potential Using Pluripotent Embryonic Stem Cell Assays and the ToxCast Library

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New approach methodologies are being explored for their ability to quickly evaluate the human toxicity potential of chemicals with less reliance on animal testing using ToxCast/Tox21 generated *in vitro* data on thousands of chemicals utilizing high-throughput screening (HTS) assays. Leveraging a high-confidence reference set (127 compounds) of prenatal developmental toxicity, we trained numerous machine learning algorithms using AC50s from the broader ToxCast/Tox21 HTS portfolio (1411 assay features) to develop a deterministic predictive model for human developmental toxicity. Within the ToxCast/Tox21 portfolio is the Stemina devTOXqp assay that measures a critical drop in the ornithine/cystine (O/C) ratio in the culture medium of H9 human embryonic stem cells (hESCs) maintained pluripotent for a 3-day exposure window to provide a predictive biomarker for human developmental toxicity potential. We obtained a positive signal on 183 (17%) of 1062 ToxCast chemicals tested to date. Balanced accuracy (BAC) of 62% was shown for 432 chemicals having any developmental toxicity evidence from studies in pregnant rats or rabbits (Toxicological Reference Database: ToxRefDB); however, BAC exceeded 77% (63% sensitivity, 90% specificity) for a subset of 127 compounds with strong evidence of developmental toxicity or non-toxicity. The winning model was L2-penalized logistic regression, with an overall 5-fold cross validation BAC of 81% (70% sensitivity, 92% specificity) against the 127-compound training set. Evaluation of the log-odds coefficients from the logistic regression model selected the hESC devTOXqp O/C ratio as the most predictive ToxCast assay feature. Finally, we trained a Bayesian logistic regression model with L2-regularization on the entire 127-compound training set to develop a probabilistic model for predicting developmental toxicity. Using ToxCast bioactivity profiles, the model predicted developmental toxicity potential of the remaining 305 chemicals with *in vivo* prenatal developmental toxicity evidence from ToxRefDB and the literature, allowing for external validation of these predictions. Overall, this model leverages hESC biology and

complementary pathways to make probabilistic predictions of developmental toxicity potential for chemicals across the broader ToxCast landscape. *This abstract does not reflect US EPA policy.*

2050 *In Silico* Prediction of Organ Toxicity: Development of *In Silico* Models from *In Vivo* Drug Histopathology Data from Regulatory Toxicity Study Reports

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Diverse activities are ongoing regarding *in silico* predictions in the regulatory context. An example is the "*in silico* Toxicology Protocols" consortium defining internationally harmonized & regulatory agreed principles of *in silico* toxicology analysis. Another example is the EMA "Reflection paper on the qualification of non-genotoxic impurities". In this context, we developed *in silico* models that were trained with findings from preclinical toxicity study reports for regulatory submissions. The sources of these *in vivo* data were the eTOX database with unpublished toxicity studies from 13 industry partners (1,947 drug candidates, 8,196 studies), approved drugs from PharmaPendium as well as other publicly available data, e.g. Leadscope database. In a first step, we focused on most important *in silico* models for regulatory decisions based on histopathology data from the main target organs liver, kidney and heart. The following compilation steps were applied to receive usable *in silico* model training datasets: Initially, the verbatim toxicity finding terms were harmonized using special ontologies. To receive model training sets with sufficient compound numbers and chemical space coverage, all primary histopathology terms were then combined to different clusters of similar toxicity mechanisms. For the most general clusters, terms were grouped into "tissue damage", "inflammatory changes", "structural alterations" or "accumulative lesions" clusters. Examples of more specific "tissue damage" cluster terms include "necrosis", "steatosis", "degeneration" etc. This resulted in many training datasets for which different modeling approaches were applied (structural alerts, fragment-based, molecular descriptor-based machine learning approaches). Models were validated and optimized by internal and external validation using Sanofi's confidential data. For example, for necrosis models (110-230 positives, 180-200 negatives) sensitivities of 76-80% and specificities of 77-78% were obtained when using fragment-based models to predict necrosis effects in liver, kidney and heart. These validation results show that by reasonable clustering of histopathology data, it's possible to develop *in silico* models with good predictivity for toxicity findings in main target organs.

2051 Improvement of Ames Test Database for Developing QSAR Prediction Models

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For predictive toxicology such as development of *in silico* predictive models and/or safety assessment using read-across approach, databases of toxicological testing are crucial. Especially GLP testing conform to the study guidelines have been categorized as "reliable without restriction" and preferred for utilization. We established a unique proprietary Ames test database containing 12,140 new chemicals based on the registration of new chemicals under the Industrial Safety and Health Act (ANEI-HOU) in Japan. Essentially studies in the database conform the study guideline of ANEI-HOU, which is practically equivalent to OECD TG471, under GLP compliance. We previously provided information of just outcome (positive or negative) with chemical structure to QSAR vendors for developing their predictive models. Recently we could obtain study reports of these registered chemicals and reviewed them of 1,743 positive chemicals enabling us to add further information, including judgement of each strain, purity of the chemicals tested, and solvent used, into the database. We also identified ambiguous data through the review and revised the judgment of 22 positive chemicals as negative or not applicable, based on a consensus of experts. Key reasons for the revisions attributed evolving interpretation of the study guideline as well as difficulty of uniform application of defined criteria on biological responses. Our results indicate that there are still gaps among testing facilities in judgement and/or interpretation even in GLP testing and suggest the need to re-evaluate actual study data when a result conflicts with growing knowledge on structure-activity relationship. Within restriction of confidentiality, our database is freely available for developing *in silico* predictive models.

2052 Optimization of Culture Conditions and Identification of Reference Chemicals for Combination Screening Using TempO-Seq and Cell Painting

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The recently released Next Generation Blueprint for Computational Toxicology at the USEPA advocates the use of broad-based high-content profiling assays as a first step for characterizing the biological activity of environmental chemicals. Two such high-throughput profiling approaches being evaluated are whole transcriptome targeted RNA-Seq (i.e. TempO-Seq) and high content imaging-based phenotypic profiling (i.e. Cell Painting), both of which can be applied to a variety of human-derived cell types. This work describes the optimization of the U-2 OS human osteosarcoma cell model for combination screening with TempO-Seq and Cell Painting. First, a time course experiment was performed in 384-well format to identify an initial seeding density (i.e. 3,000 cells/well) that would yield enough cells / well to satisfy TempO-Seq lysate requirements (i.e. $0.25x10^6$ - $2x10^6$ cells / mL lysate) and result in non-confluent monolayers for Cell Painting at 48 h, post-seeding. Next, a set of eleven chemicals with known molecular modes-of-action were screened in concentration-response mode (n = 7 concentrations, ½ log10 spacing) in order to identify a set of three phenotypic / gene expression reference chemicals for use in evaluating TempO-Seq and Cell Painting assay performance during large-scale screening campaigns. Following 24 h of treatment, each candidate reference chemical produced concentration-dependent changes in phenotypic profiles in the Cell Painting assay that were similar to those previously observed in experiments using lower initial seeding densities (400 cells / well). The benchmark concentration for onset of phenotypic changes was similar between low and high density cultures whereas benchmark concentrations for cytotoxicity were right-shifted at the higher cell density. Baseline gene expression profiling of U-2 OS cells with whole transcriptome TempO-Seq confirmed expression of the glucocorticoid (NR3C1) and retinoic acid (RARA) receptors. Well-characterized agonists of these receptors (dexamethasone and all-trans-retinoic acid, respectively) produced concentration-dependent changes in mitochondrial and endoplasmic reticulum morphology. These chemicals, plus the topoisomerase II inhibitor etoposide, were selected as reference chemicals for high-throughput profiling studies in the U-2 OS cell model. *This abstract does not reflect US EPA policy.*

2053 Molecular Docking of Putative Novichok Nerve Agent Isomers into Human Acetylcholinesterase

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Organophosphorus (OP) nerve agents owe their extreme toxicity to potent inhibition of nervous system acetylcholinesterase (AChE). Novichok agents, such as A-234, are claimed to be as much as 8 times more toxic than VX, but the structure of A-234 has not been definitively assigned. Two versions of A-234 structures have been published: one by Hoenig (HA-234) and the other by Mirzayanov (MA-234). However, neither version specifies the possible stereoisomers or protonation states of these compounds nor precisely how they interact with AChE. Moreover, although it is known that S-VX is more toxic than R-VX, due in part to its much greater inhibitory potency against AChE, it is not entirely clear which features of VX-AChE interactions confer the inhibitory advantage to the S-isomer. To test the hypothesis that at least one molecular form of A-234 has higher potency against human AChE than S-VX, we characterized OP-AChE interactions by molecular docking. Our 17 ligands consisted of 8 stereoisomers of HA-234, 4 stereoisomers and 2 protonation states of MA-234, two stereoisomers of VX, and another OP compound, echothiophate. We used covalent docking with the YASARA 19.7.20 implementation of Autodock 4.2.6 to form the trigonal bipyramidal transition state of each compound with human AChE (PDB 4M0E). This was followed by successive bond deletions, energy minimizations, and local-search docking to generate the following respective additional states for each ligand: reversible, inhibited, and 1-2 possible aged states. Assessments for each state included overall and normalized (per number of heavy atoms, Nh) binding energy, hydrogen-bond energy, residue contacts, and atom contacts. Similarities among atom contacts per residue were analyzed by generating frequency distributions and calculating the overlap (OV) among the 136 possible ligand pairs. Contrary to our hypothesis, the results did not indicate OP-AChE interactions that favored any A-234 form over either isomer of VX. Indeed, one of the most striking findings was that S-VX exhibited a higher aged-state hydrogen-bond energy per Nh (4.25 kcal/mol/atom) than any of the other ligands (1.07 - 2.73 kcal/mol/atom). Moreover, whereas the greatest OV scores (0.90 - 0.94) were between HA and MA isomers, the lowest were between S-VX and MA or HA isomers (0.38 - 0.51) as well as between S-VX and R-VX (0.41), indicating that S-VX forms a unique set of OP-AChE contacts compared to its enantiomer and the A-234 molecular forms.

2054 Quantitative *In Vitro* to *In Vivo* Extrapolation (QIVIVE) for Potential Endocrine Disruptors: Simple Generic PBTK Model versus Physiological 26-Compartment PBTK Model Including Uncertainty and Variability Aspects

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In animal-free risk assessments, effect concentrations are obtained *in vitro* and are translated to external doses (e.g. oral) by physiology based toxicokinetic (PBTK) modeling (reverse dosimetry). This approach was applied for potential endocrine disruptors - Caffeine (CAF), Ketoconazole (KET), Genistein (GEN) and Bisphenol A (BPA) to translate quantitatively lowest effect concentrations (LOEC) from *in vitro* assays to lowest observed effect levels (LOEL). Dose dependent maximum plasma concentrations C_{max} (unbound) were used to bridge *in vitro* to *in vivo*. This was modelled by 1) an 8 compartment PBTK model [1] and 2) physiological 26 compartment PBTK model (PK-Sim®[2]) for the rat. The established models were compared for predicted C_{max} to the *in vivo* C_{max} reported for various doses in the literature. Furthermore, the estimated versus measured LOELs were compared. Variability on input parameters were taken into consideration. For PBTK models 1) and 2), *in vitro* based estimated LOELs were calculated to be > 39 and 27.5, 100 and 100, 143 and 21 and 515 and 375 mg/kg bw for CAF, KET, GEN and BPA, respectively. For CAF and KET, both models predict in similar magnitude to the *in vivo*-derived LOELs. However, for GEN and BPA model 1) overpredicts the dose by a magnitude of 7-fold and 1.5-fold as compared to model 2). Furthermore, as an example, on introducing the variability on the intestinal permeability for CAF, the predicted LOEL C_{max}, varies 1.5-fold, AUC varies 1-fold and the t_{max} slows down 0.5-fold. This demonstrates that variability should be considered for more precise QIVIVE. In conclusion, this work is a significant step towards establishing full blown PBTK model using variability based QIVIVE to simulate rat TK utilizing all available *in silico*, *in vitro* and *in vivo* data for the compounds. This work attempts to determine the most appropriate assumptions to use in prospective predictions of absorption, distribution and clearance to aid chemical candidate nomination along with identifying the limitation of the bottom-up approach.

2055 Role of a Chemoinformatics Platform in Industry Research Programs and Risk Assessments for Cosmetics Ingredients

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Cosmetics Europe (CE) research activities in non-animal methods are managed by the Long Range Science Strategy (LRSS) program in the areas of systemic (toxicokinetics/dynamics), genetic and ocular/dermal toxicity. The LRSS explores the use of New Approach Methodologies to complete cosmetics safety assessments using a combination of *in silico* and *in vitro* data. To this end, a global safety assessment system, CE-TOXGPS, has been developed based on a chemoinformatics platform, integrating data and knowledge to allow for toxicity predictions and safety assessments relevant to the cosmetics industry. Safety assessment case studies that apply the SEURAT-1 safety assessment workflow and the International Cooperation on Cosmetics Regulation principles of next generation risk assessment are at the center of the system design. Two case studies are exemplified in this study that guided and demonstrated the system design and capabilities: *ab initio* safety assessment of phenoxyethanol and read-across of parabens. To allow hypothesis generation, the system provides data from COSMOS DB and IUCLID from REACH, as well as the compilation of ADME and kinetics data. In addition to the chemoinformatics features of structural searching/matching, CE-TOXGPS also offers molecular/physicochemical property calculations, mode-of-action derived profilers (DNA/protein binders, structural rules for liver and reproductive/developmental toxicity), and hepatic/dermal metabolic reactivity profiles. For skin sensitization, several prediction models from various CE members are available. Other safety assessment tools such as Threshold of Toxicological Concern (TTC) and the Read-Across-Ready table are used as evidence in the workflow. Our goal is to provide an open and extendable system that can interface with and leverage other knowledge systems such that the cosmetics industry and global regulatory bodies can share a common assessment language and paradigm.

2056 CYP Machine-Learning Models for Predicting Metabolism and Drug-Drug Interactions of Xenobiotics

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Little is known about the metabolism of organophosphate (OP) compounds (i.e. pesticides by human drug metabolizing enzymes). OP pesticide accidental exposure kills hundreds of thousands of people every year. Over the past 20 years, large quantities of *in vitro* and *in vivo* data have accumulated on human drug metabolism. Machine learning methods have been applied to many datasets in pharmaceutical and toxicological research over the past decades to enable prospective prediction and increase efficiency by minimizing testing. The availability of drug metabolism and inhibition data enables the building of predictive computational models that can aid in predicting metabolism and drug-drug interactions using molecular structure. We now describe our process for curating these data for human metabolizing enzymes from ChEMBL, PubChem and other sources, followed by the building of Bayesian and other machine learning models. For the prediction of drug-drug interactions K_i or IC_{50} models were generated using ECFP6 descriptors for CYPs including CYP1A2, 2B6, 2C9, 2C19, 2D6 and 3A4 (Bayesian Five-fold cross validation Receiver Operator Characteristics (ROC) = 0.89, 0.79, 0.88, 0.85, 0.89, 0.87, respectively). Prediction of CYP inhibition of OP compounds is critically important to identify possible drug-drug interactions leading to unforeseen toxicity. Individual CYP K_m data from databases such as ChEMBL and PubChem are remarkably limited considering the decades of primary literature on substrate metabolism and a major component of this project focuses on the manual extraction and curation of these data from the public domain to generate machine learning models. There are publicly accessible data for drugs (CypReact, drugbank.ca, bioinformatics.charite.de, etc) with the metabolizing CYPs identified via genetic polymorphisms, as well as through various experimental techniques such as human liver microsomal correlations using a phenotypic probe compounds, metabolism by recombinant CYPs and individual CYP inhibition using selective chemical inhibitors in liver microsomes. Using these data for approximately 1600 compounds, we have developed Bayesian models for all the major CYP isoforms and with the inclusion of 1000 dummy (XenoSite), negative compounds, which produced cross-validation ROCs of ~0.9. The next phase of the project will be applying these models to predict OP compounds followed by *in vitro* verification.

2057 *In Vitro* Cardiotoxicity Testing Using Video-Based Contractility Analysis and Deep Learning

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Numerous studies have demonstrated that induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs) display physiologically relevant characteristics and recapitulate aspects of patient cardiac pathology/phenotypes *in vitro*. Accurate and reliable characterization of these cells, and their response to different chemical compounds, plays a critical role in their successful utilization in drug development and safety testing. We present a novel technique for video-based analysis of iPSC-CMs, called Pulse, which combines optical flow and correlation analysis to provide reliable measurements of contractility displacement (in microns). After motion signal estimation, noisy signals are detected and rejected using a deep learning model that has been trained on many signals across a large number of experiments. Signal processing techniques are then used to generate robust and automated measurements of beating parameters such as beat rate, contraction displacement, contraction velocity, duration of contraction and relaxation. We present a case study for using Pulse in high throughput to measure contractility parameters for a panel of drugs with known cardiotoxicity profiles. iPSC-derived cardiomyocytes from CDI were cultured on standard 384-well plates. Multiple doses of 10 different compounds (Aspirin, Bortezomib, Doxorubicin, Erlotinib, Quinidine, SAHA, Sorafenib, Terfenadine, Verapamil, and a test drug) were applied, in addition to DMSO only. Brightfield videos at each well were captured by an SI8000 Imaging System with duration of 8-seconds per video, at 3 different timepoints: at baseline, and after 24 hours and 48 hours of drug exposure. Videos of beating cardiomyocytes were analyzed using Pulse. For Aspirin, no significant changes to any beating parameters were observed at either 24 or 48 hours of exposure. For Bortezomib, an anticancer therapeutic with known cardiotoxicity, Pulse shows an increase in beat rate and beat rate variation, and a decrease in contraction, duration, and prevalence of beating cells at both 24 and 48 hours of exposure. For Verapamil, a calcium channel blocker, Pulse shows a decrease in beat duration and displacement at lower doses while higher doses cause inhibition of contraction and cell death. Conversely, for Quinidine, a sodium channel blocker and hERG inhibitor, Pulse shows a significant increase in total beat duration, which is more pronounced in the

duration of relaxation. Our results demonstrate the ability of Pulse for detecting dose-dependent changes to contractility, enabling high-throughput contractility studies of cardiotoxicity.

2058 A Developmental Ontology-Based Computational Model for Mammalian Neural Tube Closure

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Computational models of biological processes are expected to revolutionize chemical safety assessment in the not too far future. Such models provide the template for establishing quantitative adverse outcome pathway (AOP) networks that define critical key events that need monitoring in *in vitro* cell models, and provide algorithms for data integration towards defining safety profiles of chemicals at the level of the intact human. This project aims at modelling mammalian neural tube closure *in silico*, as a tool for defining the related AOP network and its critical key events that need monitoring in selected *in vitro* assays. The starting point was the retinoic acid pathway, given that this morphogen plays a crucial role in cell fate, pattern formation and morphogenesis in the early mammalian embryo. We have extensively mined the developmental biology and toxicology literature to generate the gene and cell compartment interaction map including ±40 genes and 6 cell compartments and their interactions. A cascade of gene expression changes programmed in the embryo in space and time causes location-specific cell proliferation and differentiation patterns, ultimately resulting in the development of a closed neural tube from the initial neural plate. For example, notochord-derived *sonic hedgehog* drives medial hinge point formation in the neural plate, followed by dorsolateral hinge point formation by *noggin*. Neural tube closure and neural crest cell delamination are mediated a.o. by E- and N-cadherin expression driven by *Snai1* and *Slug*. Epidermis formation over the closed neural tube is induced by *Smad1*. Longitudinal neural proliferation versus differentiation is driven by gradients of *retinoic acid* versus *Wnt* rostrally and *Fgf8* caudally. We have translated this map, produced in CellDesigner software, into a three-dimensional *in silico* neural tube closure model, produced in CC3D software, which is driven by the gene expression map. This model visualizes neural tube closure, starting from a flat surface of ectoderm in which the notochord triggers formation of the median hinge points causing the first invagination of the neural plate, after which closure of the tube occurs following formation of the dorsolateral hinge points. Subsequently, the neuroectoderm and the non-neuroectoderm fuse and the neural crest cells detach and migrate away from the fusion area. This model will allow to study *in silico* the consequences for neural tube closure of model compound-induced gene expression changes detected in relevant *in vitro* assays. *This abstract does not necessarily reflect US EPA policy.*

2059 Flexfilters: A Scalable and Flexible QSAR Platform for Addressing Complex and Diverse Types of *In Silico* Safety Assessment of Chemicals

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Computational toxicity assessment techniques are currently undergoing a significant and rapid change due to the emphasis on adaptation of alternatives to animal testing. Computational prediction of a toxicity endpoint using one algorithm, one software and one model does not seem to be adequate anymore. Continuous discovery of new adverse outcomes and toxicity pathways (AOPs and TOPs) are creating new opportunities and challenges. Availability of numerous large datasets of variety of biological events caused by chemicals are also intriguing. These changes are affecting almost all involved parties: model builders, toxicity testers and regulators. Here we will describe the development of a platform to address some of these challenges. In contrast to the conventional approach, where a fixed algorithm uses one model to make a prediction, this platform relies on a set of external instructions to perform complex risk assessment tasks. For example, every descriptor in a regression model is actually treated as an instruction that operates on a chemical and returns a value. This seemingly insignificant change enables quick creation of sophisticated and different workflows, e.g. simple models with continuous or classification outcomes, logical aggregation of multiple models to reach a decision, read across methods etc. It can also perform non-predictive operations, e.g. chemical database search, imposition of domain applicability constraints, alert identification, fingerprint generation, clustering etc. It allows scalability when new data or new mechanistic knowledge become available. We will present illustrative case studies from diverse toxicity endpoints, e.g. eco-toxicity, genotoxicity, acute toxicity, skin sensitization etc.

2060 Collaborative Modeling Project for Predicting Acute Oral Toxicity

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Regulatory agencies have a pressing need to accurately assess an increasing number of chemicals for acute oral systemic toxicity potential (LD50). With the lack of *in vitro* approaches and the availability of existing LD50 data for a broad range of chemicals, *in silico* models provide an alternative to predict acute oral toxicity and bridge data gaps. NICEATM and the ICCVAM Acute Toxicity Workgroup organized an international collaborative project to develop *in silico* models for predicting acute oral toxicity. In total, 35 groups participated, submitting 139 predictive models built using a dataset of 11,992 chemicals split into training (75%) and evaluation sets (25%). Crowdsourced models were developed for five endpoints: LD50 value, US EPA hazard categories, GHS hazard categories, very toxic (LD50 < 50 mg/kg), and non-toxic (LD50 > 2000 mg/kg). Predictions within the applicability domains of the submitted models were evaluated using external validation sets, then combined into consistent consensus predictions based on a weight-of-evidence approach, forming the Collaborative Acute Toxicity Modeling Suite (CATMoS). The resulting consensus model leverages the strengths and overcomes the limitations of individual modeling approaches. The consensus predictions are fully reproduceable and performed as well as the *in vivo* acute oral toxicity assay with evaluation set balanced accuracy ranging from 0.74 to 0.84 for the four categorical endpoints predictions and an R^2 of 0.65 for LD50. The CATMoS consensus model is available via the free and open-source tool OPERA (Open Structure-activity/property Relationship App). OPERA also provides predictions for physicochemical and pharmacokinetic properties, and other toxicological endpoints with applicability domain assessments and accuracy estimates (https://github.com/NIEHS/OPERA). CATMoS predictions for the ~850k chemical structures in DSSTox are being made publicly accessible via NTP's Integrated Chemical Environment (ice.ntp.niehs.nih.gov) and US EPA's CompTox Chemicals Dashboard (comptox.epa.gov/dashboard). *This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C. This abstract does not necessarily reflect US EPA policy.*

2061 The Comparative Toxicogenomics Database: A Comprehensive View of Chemical Exposures, Mechanisms, and Biological Effects

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The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a public resource that elucidates connections between environmental chemical exposures, molecular mechanisms, and consequent biological effects. CTD provides manually curated interactions that link chemicals, genes, phenotypes, diseases and population-based exposures. Data curation using community-accepted ontologies and controlled vocabularies enables seamless integration of key external data sets and links to other public resources in the biomedical and environmental spaces. This information can be explored with user-friendly query and analytical tools to generate testable hypotheses about chemical exposures and environmentally-influenced diseases. CTD includes manually curated data for more than 16,000 chemicals, 48,000 (cross-species) genes, 5,000 phenotypes, and 7,000 diseases. We demonstrate how users can access CTD to learn about these chemicals, genes, phenotypes, diseases, or exposure studies. Integration of all five modules allows adverse outcome pathways (AOP) for systems toxicology applications to be constructed, from molecular initiating events to population-level health outcomes. We provide case studies of CTD applications for discovery including a) construction of mechanistic pathways relating air pollution exposure to cardiovascular disease using the AOP framework, and b) identification of potential health outcomes associated with vaping based on its constituent chemicals. The applications of CTD for environmental health research are broad and ever-expanding; we look forward to your feedback.

2062 Toward Mechanistic-Based Assessment: Rapid Elucidation of the Protein Binders of Diketones Using Phenotypic and Proteomic Profiling and Biomolecular Modeling

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New approach methods based on mechanistic reasoning have the potential to provide more human-relevant information for chemical safety assessment. In particular, the thermal stabilization property of ligand-bound proteins may be exploited to elucidate xenobiotic-protein interactions that may be associated to the molecular initiating events (MIEs) of an adverse outcome. Recent advances in cellular thermal shift assays (CETSA) coupled with mass spectrometry have enabled rapid identifications of the potential protein binders of a xenobiotic in intact human cells at proteomic scale. However, most environmental agents may interact with many proteins at low affinities, and the relevancy of the identified protein binders is limited by the cell models used. We have developed the Toxicity Mode of Action (MoA) Discovery (ToxMAD) Platform to address these challenges. First, we used high-throughput imaging-based phenotypic profiling (HIPPTox) to rapidly screen and identify human cell models most sensitive to a chemical of interest. Secondly, we used isothermal dose response-CETSA ($ITDR_{CETSA}$) to identify potential protein binders in the chosen cell model. Finally, we ranked all the identified candidates using two *in silico* biomolecular modeling methods: CLICK, an algorithm that compares different candidates to a ligand based on 3D-structure superimpositions of their potential binding pockets, and molecular docking that estimates the binding affinities between the candidates and the ligand. We applied ToxMAD to study diketones, a class of favoring compounds that may lead to an obstructive airway disease, bronchiolitis obliterans (BO). Using HIPPTox, we found that a lung bronchial epithelial cell line, BEAS-2B, is the most sensitive to a diketone called diacetyl. Using $ITDR_{CETSA}$, we identified 745 proteins from BEAS-2B that may bind to diacetyl. CLICK and molecular docking analysis further reduced the candidates to 56 hits, which include several known binders of diacetyl, such as L-xylulose reductase (DCXR) and aldehyde reductase (AKR1A1). Some of these identified hits may be related to the MIEs of BO. They may help us to better understand the MoAs of diketones, and search for safer replacements.

2063 Evaluation of the *In Vitro* Toxicological Profile of Ultraviolet (UV) Filters Using Tox21/ EDSP21

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Sunscreen products are composed of UV filters and formulated for different purposes, e.g., recreational use or daily facial application. The UV filters in such products reduce the dose of solar UV thereby lessening skin damage. In 2019, the Food and Drug Administration (FDA) reclassified 12 organic UV filters as Category III, most notably octinoxate, homosalate, octocrylene, ensulizole, octisalate, avobenzone and oxybenzone, citing safety "data gaps". The key safety concerns include lack of data for endocrine disruption, dermal and systemic carcinogenicity and multi-generational reproductive and developmental toxicity. Tox21/EDSP21 program, i.e., CompTox previously ToxCast, is a high-throughput screening program of chemicals from a broad background ranging from pesticides to pharmaceuticals. Tox21 and EDSP21 identify Adverse Outcomes Pathways (AOP), Key Events (KE) and Endocrine Disruption (ED). Using the CompTox website to access Tox21/EDSP21 data, all UV filters were found to have been screened in the high-throughput bioassays. These seven organic UV filters showed low activity in the bioassays with most activity detected above the range of "cytotoxic burst" as defined by Judson *et al.* (Tox Sci. 152:323-339, 2016). The pathways that were most affected were cell cycle (5 out of 7), and, to a lesser extent, the nuclear receptor pathways (2/7) and cytokine (1/7). Most activity was observed in liver and kidney-based bioassays. These seven organic filters showed weak endocrine disruption activity when tested on bioassays measuring Androgen Receptor (AR), Estrogen Receptor (ER), Thyroid Receptor and steroidogenesis activity. With the exception of oxybenzone, all activity in the endocrine assays were at concentrations 3-10 times greater than the cytotoxic burst. For avobenzone and octocrylene, AC_{50} for androgen antagonism (53uM) and estrogen agonism (18.73 uM) were 3785 and 851 times higher, respectively, than estimated plasma concentrations calculated from C_{max} measured in humans (Matta *et al.* JAMA 321:2082-2091, 2019). These data are consistent with *in vivo* animal/ human studies showing no or very weak endocrine activity. In sum, the results from this work show that organic UV filters, which have been marketed as sunscreens for decades, have relatively low activity and, by extension, toxicological potential and endocrine disruption in humans.

2064 The Preliminary Study on Single Cell Transcriptome Sequencing of Bone Marrow Hematopoietic Stem Cells in Mice by Benzene Exposure

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Benzene is a common environmental pollutant and widely used in industrial production. Continuous exposure to benzene can cause bone marrow (BM) suppression, leading to pancytopenia, anemia, aplastic anemia and even leukemia. Previous studies in our lab confirmed that the damage of hematopoietic stem cells (HSCs) was one of the causes of benzene-induced hematotoxicity. The aim of this study was to identify the differentially expressed genes (DEGs) of BM HSCs in mice exposed to benzene, and to explore the potential mechanism of benzene-induced hematotoxicity in HSCs. C57BL/6 mice were treated with corn oil or 150 mg/kg b.w. benzene for 30 consecutive days. HSCs (approximately one in ten thousand or less of bone marrow cells) were sorted by flow cytometry, and single-cell RNA-seq was applied to analyze the gene expression. Compared with the control group, the number of WBCs in Peripheral blood and the proportion of BM HSCs were decreased after benzene exposure, indicated that the mouse model of benzene poisoning was successfully established. RNA-sequencing data indicated that benzene disturbed 1514 genes by more than 2 folds in mice BM HSCs, including 400 up-regulated DEGs and 1114 down-regulated DEGs. Top 5 upregulated DEGs related to cell proliferation were Lgr5, Tnfrsf8, Dll1, Ifit3b and Tcf7, while Zfp503, Acvr1c, Prkd1, Ddr1 and Serpinb7 were downregulated. Gene ontology enrichment analysis with all DEGs found that regulation of cellular and biological process, and negative regulation of nitrogen compound or macromolecule metabolic process were statistically overrepresented. KEGG enrichment analysis revealed that many DEGs were related to signaling pathways regulating pluripotency of stem cells, Wnt and VEGF signaling pathway. Our findings reveal significant differences in HSCs transcriptomic profiles of mice BM after benzene exposure which provide novel insights into the further mechanism study of benzene-induced hematotoxicity. *This work was supported by the National Natural Science Foundation of China (Grants no. 81730087, 81573189).*

2065 Small Heat Shock Protein 27: A Critical Determinant in the Maintenance of *Drosophila* Male Germ Line Stem Cell Functions on Exposure to Cadmium Chloride

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Stem cells, like any other cells, experience different kinds of cellular stress affecting their normal functions including self renewal and differentiation. As a stress responsive pathway, alteration in heat shock proteins (HSPs) expression has been shown to affect the stem cells behavior. Maintenance of the adult male germline stem cells (GSCs) and their functions is a crucial biological process for spermatogenesis. Several studies reported the effects of cadmium (Cd) on reproductive endpoints like reduced semen quality parameters and hence failure in male fertility. However, studies involving GSCs functions under the Cd stress affecting spermatogenesis remains unknown. Therefore, the present study was designed to investigate the effects of cadmium chloride ($CdCl_2$) exposure on GSCs maintenance and in this context, evaluating the role of HSPs using *Drosophila* testes as an *in vivo* model for male germline stem cell toxicity and hence male sub fertility. Developmental exposure of $CdCl_2$ lead to significant reduction in the GSC number in $CdCl_2$ (20 µg/ml) exposed males as compared to controls. The resulting testes also showed precocious and incomplete differentiation and reduction in mitotically active spermatogonial cells along with significantly reduced reproductive performance of $CdCl_2$ exposed male flies. Furthermore, significant elevation of reactive oxygen species (ROS) level was seen in the apical region of the testes on $CdCl_2$ exposure altering small HSP 27 expression levels among various other screened HSPs. However, cell death was not induced by ROS. Genetic screens altering HSP 27 expression affected adhesion molecules at the stem-hub cells interface in the apical region of the testes on $CdCl_2$ exposure. In summary, the study infers the elevated levels of ROS resulted in altered HSP 27 expression reducing GSCs number accompanied by inappropriate differentiation leading to reduced number of sperms and eventually the fertility of male *Drosophila.* This study provides a basis of chemical stress induced dysregulation in HSP 27 expression and also suggests that small HSP 27 plays a critical role in the maintenance of germline stem cell homeostasis in Cd-induced failure in male fertility and further advocates *Drosophila* as an alternative animal model for evaluating male germline stem cells toxicity.

2066 Engineering Human Neural Organoids to Explore Impaired Neurogenesis Induced by Arsenic

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Modeling the development and function of the human brain is challenging due to the vast complexity of the organ. Recent advances in the derivation of brain-like organoids from human pluripotent stem cells (PSCs) have provided new tools to study the biology of the human brain. These 3D *in vitro* models have the potential to enhance our understanding of the mechanisms of developmental neurotoxicity during the early stages of neurogenesis and offer a cost-effective approach for assessing chemical safety. Here, we have used PSC-derived embryoid bodies (EBs) from 384-well plates to develop neural organoids for chemical developmental neurotoxicity testing. There is overwhelming evidence that environmental factors play a role in the development and progression of a host of central nervous system disorders. Arsenic (As) is a widespread environmental contaminant. Human exposure to As occurs mainly through ingestion of contaminated food or water. Exposure to inorganic As is associated with developmental neural diseases; however, the mechanisms of As on developmental neurotoxicity are not well-defined. We used 3D EBs to recapitulate events involved in early embryogenesis and neurogenesis. We found that a 7-day exposure to a human-relevant, non-cytotoxic dose (0.5 μM; 35 ppb) of As showed irregular early neural rosette-like structure formation and increased ectoderm differentiation within the EBs through upregulated expression of genes PAX3, PAX6, SOX1, COL2A1 and the Notch signaling pathway, which all play critical roles in early embryonic development. During neural induction stage (day 7) of neuron organoid formation, As exposure increased expression of neural progenitor cell marker genes NESTIN and PAX6. Histologic assessment of day 40 control neuron organoids validated the presence of neuroepithelial cells forming neural rosette-like and neuropil structures. Immunohistochemistry showed regions of vimentin+ astrocytes and nestin+ neural stem cells in the organoid structures. The neural rosette-like structures were disrupted in day 40 neural organoids with As exposure. Using the neural organoid 3D model described here can provide valuable insights into the cellular events and molecular mechanisms to address adverse outcome pathways associated with As-induced developmental neurotoxicity.

2067 Impact of Trisomy 21 and Pesticide Exposure on Cell Fate Lineage Specification in Directed Neural Differentiation

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Down syndrome (DS) is a complex genetic insult caused by triplication of chromosome 21. The ubiquity of cognitive deficits in DS has made structural and cellular changes in the brain the focus of much research effort. However, no prior studies have utilized DS induced pluripotent stem cells (iPSC) to assess the effect of trisomy 21 and environment on embryoid body formation and neural lineage specification. Accumulating evidence has shown that phenotypic features of the DS brain originate in early developmental stages. We aim to study the interface between genetic mechanisms, developmental processes, and environmental exposures to elucidate the stages where normal patterns of development may diverge in DS. Here we show neural induction by monolayer culture system and embryoid body formation - two processes that generate neural progenitor cells (NPC). NPCs are multipotent, and generate the major cell types of the central nervous system (CNS). In our preliminary experiments involving an isogenic model of euploid and DS iPSCs, we observed differences in EB size, as well as several neural differentiation markers, such as TuJ1 (neurons) and GFAP (glial cells), to be upregulated in DS. Furthermore, pluripotency (OCT4) and mesodermal (brachyury) markers remain elevated in DS during neural induction in EB formation and monolayer culture. Trisomic iPSCs may be biased towards particular germ lineages or stem cell self-renewal despite directed differentiation. These observations, along with the lack of proper neural rosette formation in DS iPSCs, suggest accelerated neural and glial differentiation, prolonged pluripotency, and reduced progenitor cell development. As the developing brain is particularly sensitive to toxic environmental exposure, the pesticide, maneb (MB), was incorporated to identify critical developmental periods and pathways that are vulnerable in the context of trisomy 21. Our preliminary data demonstrate that DS cells display greater toxicity to the fungicide, MB. In the presence of MB, DS EBs showed altered morphology and caspase activity compared to their euploid counterparts. Together, these data indicate that variability in DS phenotypes may not be a direct product of trisomy 21, but rather a consequence of an interaction between genetic and environmental factors.

2068 Protective Role of Mesenchymal Stem Cells and Mesenchymal Stem Cell-Derived Exosomes in Cigarette Smoke-Induced Mitochondrial Dysfunction

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Cigarette smoke (CS)-induced lung inflammation and chronic obstructive pulmonary disease (COPD) involves mitochondrial dysfunction. Mesenchymal stem cells (MSC) and MSC-derived exosomes (EXO) are reported to show their therapeutic effects in animal models of inflammation/injury. We hypothesized that MSC and EXO combination (MSC+EXO) treatment may have protective effects against acute CS-induced lung inflammation and mitochondrial dysfunction compared to individual treatments (MSC or EXO alone). EXO were characterized by Western blotting, tunable resistive pulse sensing (qNano) and transmission electron microscopy. Mitochondria reporter mice (mt-Keima and mito-QC) were exposed to air or CS for 10 days. mt-Keima mice were treated with intraperitoneal injections of MSC or EXO or MSC+EXO for 10 days. Total cell counts and differential cell counts were analyzed using cellometer and flow cytometry respectively. Pro-inflammatory mediators in bronchoalveolar lavage fluid were measured by ELISA. Mitochondrial, DAMPs, inflammation and mitophagy markers were further analyzed by Western blot analysis, qPCR and confocal microscopy in acute CS exposed mouse lungs. Seahorse flux analyzer was used to measure the oxidative-phosphorylation (OXPHOS) in the BEAS2B cells and mMSC co-culture experiments. CS exposure increased the inflammatory cellular infiltrations in the lungs of the mt-Keima mice, while treatments showed varied degrees of protection. There were no changes in the mitophagy proteins like Pink1 and Parkin, which was also found using the mito-QC mice. Acute CS exposure increased the protein abundance of DRP1 (mitochondrial fission), S100A4 and S100A8, HMGB1, RAGE and AGE (DAMPs markers). MSC+EXO treatment increased the gene expression of *mfn1, mfn2* and *opa1* (mitochondrial fusion) and *rhot1* (mitochondrial trafficking). BEAS2B+MSC co-culture showed protective response against the CSE-induced mitochondrial respiration (OXPHOS) confirming the beneficial effect of MSC to lung epithelial cells. MSC+EXO treatment showed attenuation of selected inflammatory and DAMPs markers such as MMP9, S100A4 and HMGB1. MSC+EXO treatment may act against these early events caused by CS exposure owing to its anti-inflammatory and other mitochondrial transfer mechanisms. *Supported by the NIH R01 HL135613, R21 ES028006, R01 HL133404, and R01 HL137738.*

2069 Fluid from Popular Electronic Cigarettes Negatively Impacts Early Prenatal Development as Assessed Using Human Embryonic Stem Cells as a Model

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Electronic cigarettes (ECs) are often recommended to pregnant women as an alternative to smoking. However, their effects on embryonic and fetal health have not been rigorously assessed. Recently, vaping-associated pulmonary illness (VAPI) has raised awareness regarding possible harm that ECs may cause. ECs expose the user and conceptus to nicotine, flavor chemicals, solvents, metals and reaction products. This project examined the effect of fluid from two popular ECs and pure nicotine on early development using H9 human embryonic stem cells (hESC) taking a "toxicology in a dish" approach. hESC were cultured on Matrigel and maintained in mTeSR Plus medium. To examine the effect of e-liquids on attachment, cells were plated into 1% dilutions of JUUL Virginia Tobacco, JUUL Classic Menthol, Vuse Original and Vuse Menthol e-liquids, allowed to attach for 24 hours, then the number of attached colonies was determined in micrographs of nine fields of view/treatment. Attachment of hESC was inhibited in both JUUL and Vuse treatments. The impact of the e-liquids and nicotine on cell proliferation was also studied. hESCs were plated on 24-well plates, and after a 24-hour attachment period, treated with various concentrations of nicotine or e-liquids. Time-lapse images were collected over 48 hours in a BioStation CT. MTT assays were performed to determine the effects of treatment on mitochondrial reductases. Images were analyzed using CL-Quant to obtain growth rates. Proliferation of cells exposed to e-liquids was not affected in the 0.1% and 0.3% e-liquid treatments, but was impaired in the 1.0% treatments, and cell death was seen in the 3% treatment groups. Cells exposed to the nicotine concentrations in 0.1%-1% e-liquids (0.034-0.160 mg/ml) showed growth rates like the control. Exposure to 1.01-1.83mg/ml nicotine (found in 3% e-liquids) resulted in cell death. The MTT assay results showed that 1% e-liquid impaired mitochondrial reductase for each flavor. Nicotine MTT results showed that lower concentrations stimulated mitochondrial reductase but higher concentrations were toxic. Failure to attach to substrates and reduced proliferation could have detrimental effects during early human development. Based on these data, women should avoid using ECs during early pregnancy when the embryo is most susceptible to toxicants.

2070 Toxicogenomic Profiling of Human Neural Progenitor Cells Exposed to Alternative Flame Retardants

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Due to their environmental persistence and suspected ability to cause developmental neurotoxicity (DNT), polybrominated diphenyl ether (PBDE) flame retardants (FRs) are gradually being phased out of consumer products. Organophosphorous FRs (OPFRs) are being introduced as replacements. Alarmingly, select alternative FRs and/or their metabolic products are widely-detected in human maternal blood, urine, and placental tissue. While the developmental health effects of exposures to these compounds remains unknown, initial *in vitro* assessments suggest that select compounds may induce developmental toxicity at similar potencies as legacy FRs. Human embryonic stem cell (hESC) models are promising tools to evaluate the ability of chemicals to cause DNT. Previously, we demonstrated that hESC-derived neural precursor cells (NPCs) are sensitive to PBDE-induced DNT, altering cell function and global gene expression. In subsequent analyses, we expanded our assessments to include 12 alternative FRs. We discovered 9 of the 12 tested FRs (0-30μM) induced significant cytotoxicity, reducing cell viability and increasing cell death in NPCs. To identify possible common mechanisms underlying FR toxicity, we performed sequential window acquisition of all theoretical mass spectra (SWATH-MS) proteomic profiling of NPCs exposed to BDE-47 or one of three FRs identified to be as potent as BDE-47: IPP (isopropylated phenyl phosphate), EHDP (2-ethylhexyl diphenyl phosphate), or F550 (Firemaster 550) at 3 or 10 μM. In total, we identified 2,511 unique proteins in all NPC samples (n=30). Significant alterations in protein abundance were observed with all four compounds ($p<0.05$; absolute fold change > 1.2) as compared to the vehicle control (0.1% DMSO): BDE-47 (75); IPP (99); EHDP (54); and F550 (70). In general, proteins were altered in a concentration- and FR-dependent manner. The largest overlap in dysregulated proteins was observed between the two OPFRs, IPP and EHDP (9 proteins). Functional analysis of differentially expressed proteins suggested potential common mechanisms across FRs, including altered mitosis (BDE-47, F550), hormone response (EHDP, F550) or organonitrogen metabolism (IPP, EHDP, F550). Our results suggest alternative FRs are neurodevelopmental toxicants in a hESC model of neurogenesis. They induce concentration-dependent toxicity and alter protein expression in DNT-relevant pathways. Future studies will examine the correlation between proteomic and transcriptomic FR signatures, and the functional relevance of proposed targets. *Supported by NIH/NIEHS R00ES023846.*

2071 Establishing Cell Painting for Morphological Studies of Toxicant Effects in Human Mammary Cells

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High Content Imaging (HCI) uses phenotypic screening to study whole cells and cellular components by simultaneously assessing multiple phenotypes in complex cell populations. Cell Painting, a phenotypic HCI assay, uses automated fluorescence microscopy to simultaneously measure 8 cellular components via six fluorescent stains which are imaged in 5 channels. The cellular components include DNA, RNA, endoplasmic reticulum, mitochondria, F-actin, golgi apparatus, and plasma membrane. Cell Painting was shown to be effective in quantifying subtle perturbations in cellular and sub-cellular features to identify mode of action for drugs and chemical stressors and to find genes implicated in disease onset and development. The goal of this project was to establish the Cell Painting method to study the effects of environmental toxicants on human mammary cells to assess potential carcinogens and stem cell toxicants. As a proof of principle, we tested the effects of cadmium on the non-tumorigenic human mammary cell line MCF10A. Cadmium is a putative metalloestrogen which dysregulates mammary stem cell biology at physiologically relevant doses. MCF10A cells were treated with cadmium doses ranging from 20nM to 10uM for 48 hours, subjected to Cell Painting, and images were captured via a CellInsight CX5 HCI microscope. Image data were quantified in an unbiased manner using CellProfiler software, which yielded approximately 3000 distinct morphological measurements per cell, including staining intensity, texture, and localization. Dose-response effects across various morphometric features were quantified in R and BMDExpress. CellProfiler analysis revealed dose-dependent alterations in cellular morphology, both nuclear and cytoplasmic. BMDExpress analysis identified multiple changes in granularity and intensity of DNA staining and granularity of cytoplasmic golgi and actin staining between 750 nM and 1 uM cadmium exposure. Ongoing work is quantifying alterations in cell morphology across a range of chemical exposures at the single cell level to understand cellular heterogeneity in response to toxicant effects. Cell Painting is a cost-effective, easily customizable technique that can identify the morphological effects of environmental toxicants and could aid in deducing modes of action and pathways involved in development of breast cancer.

2072 Hepatocytes Derived from NASH iPSC Donors Provide a Valuable Platform for Disease Modeling and Drug Discovery

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Non-alcoholic fatty liver disease (NAFLD) affects 30 to 40% of adults and about 10% of children in the US. About 20% of people with NAFLD develop non-alcoholic steatohepatitis (NASH), which may lead to cirrhosis, onset of hepatocellular carcinoma and is expected to become the leading cause of liver transplantation in the near future. Development of NASH may be exacerbated by toxic insults to the liver. Human induced pluripotent stem cells (iPSCs) derived from NASH patients are a valuable tool for studying NASH pathobiology at the cellular and molecular level and to improve drug target screening. We have developed a novel hybrid two dimensional/aggregate protocol to generate cryoperservable hepatocytes using a panel of iPSCs from NASH patients and healthy donors. iPSCs from both sets of donors demonstrated a rapid decline in pluripotency genes *POU5F1* and *NANOG* at the onset of definitive endoderm (DE) differentiation. End stage DE cells coexpress high levels of CXCR4 and CD117 and, when differentiated further along the hepatic lineage, developed >90% purity of hepatic markers alpha-1 antitrypsin (AAT), asiaglycoprotein 1 (ASGPR1). End stage hepatocytes also developed expression of albumin. Importantly, both cryopreserved DE and hepatoblasts also successfully differentiated to mature functional hepatocytes demonstrating ~2-fold induction of CYP3A4 activity in response to rifampicin. Additionally, hepatocytes from healthy and NASH donors demonstrated lipid accumulation upon fatty acid (FA) treatment. Interestingly, hepatocytes derived form one of the NASH donors demonstrated spontaneous lipidosis mimicking hallmarks of NASH hepatocytes. Co-culture of hepatocytes along with iPSC derived macrophages and mesenchymal stem cells from healthy and NASH donors will enable generation of liver organoids that would be considerably valuable for understanding NAFLD/NASH biology, identifying novel targets for treatment, and will improve safety assessments of the potential drugs aimed at treating the disease.

2073 High-Throughput Anisotropic Human iPSC-Derived Cardiomyocyte Cultures Display Differential Response to Chronic Oncology Compound-Related Toxicity

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Cardiac toxicity derived from chronic exposure of oncology treatments can be a concerning side effect of those therapies. The ability to better detect and dissect chronic cardiac toxicities in a human and tissue-specific *in vitro* high throughput system would enable a better understanding of the mechanisms underlying those toxicities, potentially leading to strategies to mitigate or eliminate them. Human induced Pluripotent Stem Cell-derived Cardiomyocytes (hiPSC-CMs) have been employed successfully in the detection of chronic cardiac toxicities arising from several independent oncology drugs. Nonetheless, current remaining challenges under consideration for the hiPSC-CM system are sub-ideal cardiomyocyte geometry, sub-cellular structural organization, and coherent contractile behavior in standard monolayer cultures. Independent Bioengineering approaches have shown improvements in aspects of hiPSC-CM physiology *in vitro*, however those approaches have limited scalability and thus are not amenable to high throughput screening. hiPSC-CMs cultures passively aligned on a high throughput platforms have been shown to display physiologically-relevant features, including more physiological cellular geometry, coherent unidirectional contraction, cardiac cell junction re-modeling, and improved calcium handling. To evaluate whether the changes induced by this platform translated into differential responses to compounds previously liked to chronic cardiotoxicity, high throughput calcium flux and viability assays were performed on hiPSC-CMs cultured in standard high throughput screening cell cultureware or anisotropic 384-well plates. hiPS-CMs were interrogated with 14 compounds previously linked to chronic cardiotoxicity across a 7-point concentration range. Interestingly, compounds like Doxorubicyn and Vandetanib displayed a differential response in anisotropic conditions, with more evident arrhythmic behavior detected at lower concentrations than in standard monolayer cultures. Additionally, Vandetanib induced the occurrence of severe early afterdepolarizations (EADs) that weren't observed in standard culture conditions aside from the highest doses tested. Simultaneously, no evident ab-

normal arrhythmogenic behavior or EADs were observed for vehicle control (DMSO) treatment or Aspirin, a negative control compound. Taken together, the observations from this study indicate that anisotropic high throughput hiPSC-CM cultures showed better sensitivity and resolution over the progression and severity of pro-arrhythmic events.

PS 2074 Investigating the Impact of Trisomy 21 on Xenobiotic Biotransformation Using Induced Pluripotent Stem Cell-Derived Hepatocyte-Like Cells

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Individuals with Down syndrome (DS, Trisomy 21) experience an array of comorbidities in addition to intellectual disabilities including type 1 diabetes, hypothyroidism, obesity, leukemia, inflammation and Alzheimer's disease. These comorbidities are of great relevance to this study due to the fact that genetics, disease states, and cofactor levels can greatly impact xenobiotic biotransformation. To investigate the impact of DS on xenobiotic biotransformation, we utilized hepatocyte-like cells (HLCs) derived from two isogenic induced pluripotent stem cell lines (iPSCs). These cell lines, C3 (CTRL) and C2 (DS) are ideally suited to investigate the impact of trisomy 21 on drug metabolism as they are derived from the same fibroblast cell line, AG06872; therefore, any differences observed from this study are exclusively due to the trisomy of chromosome 21. HLC phenotype was assessed by gene expression (i.e. HNF4α), glycogen synthesis and cytochrome P450 (CYP) activity. We then administered agonists for xenosensor transcription factors, like the aryl hydrocarbon receptor (AhR) and constitutive androgen receptor (CAR), and analyzed downstream drug metabolizing enzyme (DME) expression. HLCs were treated with benzo[a]pyrene (BaP, AhR agonist) for 24 hours to induce downstream Phase 1 DME (ALDH3A1, CYP1A1, CYP1B1) and Phase 2 DME (GSTA1) expression. After 24 hours of BaP (10μM) stimulation, we detected variations in DME gene expression in DS compared to CTRL with ALDH3A1 (1 to 1.9 fold change in CTRL vs DS), CYP1A1 (6.0 to 5.4), CYP1B1 (1.6 to 2.0) and GSTA1 (1.3 to 1.8). To induce downstream CAR gene expression, HLCs were treated with 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)oxime (CITCO, CAR agonist) for 24h and Phase 1 DME (CYP3A4, CYP2B6) expression determined. Following 24 hours of CITCO (1μM) treatment, we saw a drastic increase in CYP3A4 in DS compared to CTRL (1.19 to 11.9 in CTRL vs DS). These studies highlight the inherent differences that having the presence of trisomy 21 has on xenobiotic biotransformation and induction of Phase 1 and 2 DME. Further, these data show that DS individuals do not metabolize xenobiotics in a manner similar to euploid, indicating that additional characterizations are necessary to effectively understand drug and toxicant metabolism in this unique genetic background.

PS 2075 Single Cell RNA Sequencing Reveals Differences in Transcriptomic Profiles of Normal Mammary Cells between African American and European American Women

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In the US, women of African ancestry have significantly worse breast cancer outcomes. In particular, African American women are two to three times more likely to develop the most aggressive subtype, triple negative breast cancer (TNBC). The biological basis for these disparities remain elusive. Mounting evidence points to an important role for stem cells in breast cancer, and studies have shown that normal mammary stem cell number is associated with genetic and environmental risk factors. These findings suggest that differences in stem cells may influence breast cancer incidence, survival, and even racial disparities in cancer. The goal of this study was to characterize differences in normal breast stem cell biology between women of African and European ancestry to provide insight into the potential role of stem cells in breast cancer disparities. Breast punch biopsy samples from healthy, nulliparous women were obtained from the Susan G. Komen normal tissue bank and matched on age, BMI, and days since last period. 8 African American (AA) and 8 European American (EA) tissues were successfully established in conditional reprogramming (CR) culture conditions which induce rapid expansion and dedifferentiation of primary cells. All established samples were profiled using single-cell RNA sequencing (drop-seq) in order to characterize differences in cell type proportions and cell states. Unbiased clustering revealed that post-CR, cells both mix and form distinct clusters by individual and by race. All samples retained luminal and myoepithelial mammary cell populations which varied greatly by individual, with AA samples having a higher overall proportion of myoepithelial cells. AA samples were enriched for an embryonic stem cell gene expression signature, particularly in the myoepithelial population. Comparing gene expression between AA and EA samples revealed 135 differentially expressed genes (DEGs) in the luminal population alone, 276 DEGs in the myoepithelial population alone, and 41 overlapping DEGs in both populations. Of particular note are *S100A11* and *KRT17*, found to be higher in AA myoepithelial cells and known to be involved in differentiation and proliferation. Ongoing work is assessing the response of these cells to environmental stressors. We report that normal mammary cells from AA and EA women have differences in cell type proportions, transcriptomic profiles, and stem cell gene expression. These findings provide new insights into how differences in normal stem cell biology may impact breast cancer disparities.

PS 2076 Generation of iPSC-Derived Basal Cells and Differentiation into a Pseudostratified Lung Airway Model on Air-Liquid Interface

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The conducting airways of the lungs are constantly exposed to the damaging effects of pollutants, gasses and nanoparticles (NM) present in the atmosphere. Various chemical compounds used as medication can also have an adverse effect on the respiratory system. It is of particular interest to understand and model those interactions as part of risk assessment evaluations in the process of drug discovery. Current *in vitro* models rely on the availability of primary lung epithelial cells or the use of immortalized cell lines, which poorly represent human biology, highlighting the need for a better model system. In3 project (Marie Curie - ITN) is a collaboration between industry and academia with a common goal of development and utilisation of *in vitro* and *in silico* tools for human chemical and NM safety assessment. Our objective is to utilise iPSC technology and generate a conducting airway epithelium model that would then be used with the in3 chemical compound set for mechanistic and kinetic analysis. We have developed a new strategy to isolate iPSC-derived basal like cells of high purity from a mixed population of lung progenitors by integrating several approaches used in primary bronchial airway cell culture. Those cells are positive for lung basal cell markers cytokeratin 14, NGFR, Integrin alpha 6 and ΔNp63 and can be expanded for several passages while maintaining their multipotency. When those cells were differentiated on an air-liquid interface, they formed tight junctions and a structured pseudostratified epithelium, strikingly similar to *in vivo* like airway. The iPSC derived airway-contained functional basal cells, goblet cells, club cells and ciliated cells, confirmed by immunohistochemistry. A mucus layer was formed on the apical side and beating cilia could be observed on bright field microscopy. Preliminary exposure to chemicals relevant for inhalation toxicology gave us encouraging results: the cells were treated with chemicals such as cigarette smoke condensate, cerium oxide nanoparticles, busulfan and others for 24h and the activation of toxicological pathways was assessed by qPCR. This is the first functional iPSC derived lung airway model on an air-liquid interface, which offers many advantages over the alternative existing models which is very exciting, further characterisation will help us understand the full potential of the model for toxicology and drug discovery.

PS 2077 An *In Vitro* Bronchosphere Model to Assess Respiratory Toxicity

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In vitro model systems to assess potential toxicity of pollutants such as diesel exhaust and nanoparticles in the respiratory tract are limited to primary airway or lung epithelial cells and non-differentiated, often cancer-derived, cell lines. The purpose of the studies described here was to develop a more representative model of human airways utilizing airway basal cells which take on a 3D structure, called "bronchospheres" when maintained in a gel matrix, allowing for high throughput toxicity screening of chemicals/particles. Furthermore, the culture conditions were optimized to improve the differentiation of basal cells to goblet/ciliated cells, which is crucial for unraveling the adverse effects of pollutants on the resiliency and homeostasis of the lung airway. Normal human bronchial epithelial basal cells were purchased and cultured/expanded through 3 passages using standard methods. Passage 2/3 cells were seeded in Matrigel® with medium optimized for their differentiation. After 14 days, these cells formed spheroids with a clearly-observable lumen, accompanied by the apoptosis of cells in spheroid interior. Furthermore, gene expression of markers for goblet cells, *MUC5B*, and that for ciliated cells, *FOXJ1*, were detected in Day 14 bronchospheres, with their absence of expression in the basal cells. Passage 2 and 3 cells had similar if not identical responses to these conditions, with number and size of the bronchospheres essentially identical for each. Initial seeding density of the cells was optimized at 2400

cells/cm^2. These bronchospheres, with a 3D structure, could represent an improved *in vitro* model system to more accurately reflect the *in vivo* responses of these cells to inhaled deleterious substances. When further developed and validated, they could provide a robust high throughput method for screening for toxicity of chemicals/particles, as well as for facilitating mechanistic study on the association between environmental pollution and lung disease, and eventually reduce/replace the use of *in vivo* animal studies for such testing.

2078 Human Induced Pluripotent Stem Cell (hiPSC)-Derived Retinal Model Containing Microglial Cells as a Platform for Toxicology Studies

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Microglia (MG) are the primary tissue resident immune cells in the retina. They are essential for normal development by regulating neuronal survival and synaptic pruning. In the adult retina, they regulate homeostasis by maintaining synaptic structure and function. Under pathological conditions, MG can mediate adaptive responses within the retina following injury. In addition, MG can trigger neurodegeneration exacerbating the effect of the disease making it a potential therapeutic target. Retinal organoids (RO) derived from hiPSCs provide a human physiologically relevant platform to study retinal development, disease modelling and compound screening. However, due to the differences in their developmental origins, MG and retina do not arise under the same differentiation conditions. We developed a differentiation protocol for deriving MG from hiPSCs. The cells expressed key developmental markers CX3CR1 and IBA1. In addition, they were functional with >90% of cells phagocytosing fluorescent beads. In parallel, we differentiated hiPSCs to RO using our established protocol. We assessed their development by confirming the expression of key markers, including Recoverin, HuC/D, AP2α and Prox1. To enhance our retinal model, we incorporated hiPSC-derived MG and tested their retinal invasion capacity and function in response to agents causing retinal degeneration, namely chloroquine and moxifloxacin; and lipopolysaccharide (LPS) that is known to initiate pro-inflammatory response. Our results showed that LPS and chloroquine treatment led to an increased secretion of pro-inflammatory and chemotactic factors. LPS increased secretion of IP-10 from 59.2 ± 1.5 to 108.4 ± 5.05; MIP-1α from 37.0 ± 1.9 to 129.6 ± 1.4; MIP-1β from 83.6 ± 2.3 to 170.5 ± 11.6; and IL-6 from 1.4 ± 0.1 to 4.3 ± 0.2 (pg/ml). Treatment with chloroquine increased secretion of MIP-1α from 37.0 ± 1.9 to 82.6 ± 6.6 and MIP-1β from 83.6 ± 2.3 to 121.9 ± 4.7 (pg/ml). Moxifloxacin treatment did not induce an increase in cytokine release which is in line with its role in reducing MG activation and having an anti-inflammatory effect. Our results confirmed that MG cells co-cultured with RO are able to respond to known toxins and stimulants thereby providing a tissue structure with greater physiological relevance to the *in vivo* human retina which can be used for compound screening and disease modelling.

2079 Highly Prescribed Antipsychotics Perturb Cholesterol Biosynthesis in hiPSC-Derived Human Cortical and Dopaminergic Neural Precursors

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Smith-Lemli-Opitz syndrome (SLOS) is a rare, autosomal recessive developmental disorder whose phenotype encompasses a wide spectrum from craniofacial malformations to severe intellectual disability. SLOS patients carry mutations in the gene that codes for 7-Dehydrocholesterol reductase (DHCR7), an enzyme that converts the highly oxidizable lipid 7-dehydrocholesterol (7-DHC) to cholesterol, and thus these patients present with elevated 7-DHC/Cholesterol serum ratios. Both preclinical and clinical evidence has established unimpaired DHCR7 function as essential for proper neurodevelopment, as an accumulation of 7-DHC has been reported to negatively affect neuronal differentiation, proliferation, and viability. Recent studies have demonstrated that highly prescribed antipsychotics such as Aripiprazole and Cariprazine have off-target inhibitory effects on DHCR7 and lead to elevated 7-DHC levels *in vitro* models and in sera of patients on these medications. To determine the impact of these and similar compounds on early human neurodevelopment, we assessed their effects on cholesterol biosynthesis precursor profiles in human-induced pluripotent stem cell (hiPSC)-derived neural precursors (NPCs). hiPSC-derived day 13 cortical and dopaminergic NPCs were exposed to increasing concentrations (1-1000 nM) of Aripiprazole and Cariprazine for 48 hours. Lipid extracts were analyzed via Liquid Chromatography-tandem Mass Spectrometry (LC-MS-MS). Both cortical and dopaminergic cultures exposed to Aripiprazole and Cariprazine demonstrated a dose-dependent increase in DHCR7 substrates and a corresponding decrease in DHCR7 products. Cariprazine potently inhibited DHCR7 at the lowest dosage (1 nM), a significant finding as plasma concentrations of the drug and its metabolites can persist above 100 nM for many months in humans. Interestingly, while the small molecule inhibitors dramatically altered sterol profiles, no cytotoxicity was observed at any dosage. These findings support the hypothesis that perturbed cholesterol biosynthesis may mediate the teratogenic effects of prenatal exposures to antipsychotics, especially as similar cholesterol precursor profiles are observed in SLOS, a developmental disorder affecting the very same enzyme.

2080 Assessing DDT/DDE in Alzheimer's Disease Pathways in Human Stem Cell-Derived Neurons Carrying APOE Gene Variants

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The E4 allele of the Apolipoprotein E (*APOE*) gene contributes the greatest single genetic risk for late-onset Alzheimer's disease (LOAD), but it is also affected by factors including age and environmental exposure. The pesticide dichlorodiphenyltrichloroethane (DDT) has been identified as a risk factor for LOAD due to its persistent bioaccumulation and elevated serum levels of the DDT metabolite, dichlorodiphenyldichloroethylene (DDE) of AD patients even after it was banned in the US over forty years ago. Previous results found that there were significant interactions between serum DDE levels, *APOE* genotype and cognitive dysfunction, with *APOE* E4 genotype and higher DDE levels being associated with worsened cognitive function. Mechanistically, our previous experiments found significant increases in amyloid precursor protein (APP) levels in differentiated SH-SY5H cells and primary mouse hippocampal neurons after DDT/DDE exposure. To quantify this relationship in human neurons, an appropriate model system needs to be generated. We prepared induced excitatory neurons (iNs) by reprogramming two isogenic induced pluripotent stem cell (iPSC) lines, a heterozygous APOE E3/E4 and an engineered line carrying frameshift mutations in both alleles (APOE-null). A 24 hour treatment of APOE-null neurons with 1 μM DDT increases APP levels on Western blots by approximately 50% compared with vehicle control. To test interaction with APOE, neurons will be cultured in the presence of conditioned medium containing APOE-e2, e3, or e4 and/or oligomeric amyloid beta collected from lentiviral-transduced HEK293 cell lines to mimic conditions of Alzheimer's disease. Utilizing this novel human neuron model, we examine direct effects of DDT and DDE on neuronal function and indirect effects on AD markers. The combination of electrophysiologically-mature human neurons, addition of APOE and/or amyloid toxic signals, and environmental toxicants such as DDT/DDE represents a novel approach to modeling AD.

2081 Development of a Workflow for Multi-Modal Assessment of Functionally Matured Human iPSC-Derived Cardiomyocytes Using Calcium Imaging and Impedance Readout

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Non-cardiac drug induced functional changes in cardiomyocytes are one of the common causes of attrition during preclinical and clinical drug development. Therefore, understanding drug-mediated modulation of cardiac electrophysiology and contractility, which are important hallmarks of cardiomyocyte function are critical from both a therapeutic as well as safety/toxicity angle. Even though human iPSC-derived cardiomyocytes (hiPSC-CMs) have been validated as a suitable model for assessment of contraction (Scott C. et al, 2013), their fetal-like phenotypes, such as immature contractile apparatus and the calcium handling mechanisms have hampered full utilization. In order to address this limitation, we developed a workflow where long-term electrical pacing was applied using the xCELLigence RTCA e*Pacer* to enhance functional maturity of hiPSC-CM (paced cardiomyocytes), as assessed by reversal of negative force-frequency relationship, a hallmark of immature phenotype of hiPSC-CMs. The paced cardiomyocytes were then treated with a panel of contractile modulators, including both positive and negative inotropic compounds with different mechanisms of action and assessed for calcium dynamics via calcium sensitive dye and for contractility via impedance, respectively. Our data shows that isoproterenol, milrinone and BayK 8644, which are positive inotropic compounds increased the force of contraction by mobilizing calcium and significantly increasing the amplitude of calcium transient (Ca-Amp) and impedance/beating amplitude (B-Amp) in paced car-

diomyocytes but not in non-paced (CTRL) cells. Omecamtiv mecarbil (OM), a positive inotrope increased cardiac contractility as measured by impedance but did not show any impact on intracellular calcium concentration in both paced and non-paced cardiomyocyte. Isradipine, a L-type calcium channel blocker and negative inotrope, decreased both Ca-Amp and B-Amp in both paced and non-paced cells. In summary, our data suggests that chronically paced hiPSC-CMs could be a more improved model system for cardiac contraction assessment compared to standard (non-paced) hiPSC-CMs. The combination of calcium transient and impedance readout for cardiac contractile assessment can provide more mechanistic insights for drug induced changes to cardiomyocyte function.

PS 2082 Toxicological Assessment to Pain-Related Compounds in Cultured Human iPS Cell-Derived Sensory Neurons Using CMOS-MEA System

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Electrophysiological assessment using human induced pluripotent stem cell (hiPSC)-derived sensory neurons are expected to predict the pain-related toxicity of drugs and the *in vitro* alternatives to reduce the number of animal tests. In this study, we aimed to evaluate the electrophysiological responses against pain-related molecules including anti-cancer drugs in cultured hiPSC-derived sensory neurons using multi-electrode array (MEA) and CMOS-MEA with high temporal and spatial resolution, in which is possible to measure axon and dendrite conduction velocity of single neuron. hiPSC-derived sensory neurons were cultured on MEA and CMOS-MEA chips, and the electrophysiological evoked responses against capsaicin, menthol, AITC, anti-cancer drug oxaliplatin were detected dose dependently. We also confirmed that these responses are the responses via TRPV1, TRPM8, and TRPA1 receptors. The responses to oxaliplatin are consistent with *in vivo* report. We also detected the change of axon conduction velocity and identify the neurons having high sensitivity reaction. MEA and CMOS-MEA measurement coupled with hiPSC-derived sensory neurons is useful to assessment to pain-related compounds.

PS 2083 Prediction Methods for Seizure Liability and MoA of Drugs Based on the Electrophysiological Activities of hiPS Cell-Derived Neurons

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Human iPSC-derived neurons are expected to be applied to toxicity evaluations in nonclinical studies and drug screening. Microelectrode array (MEA) measurement system is suitable to evaluate the neuronal electrophysiological responses to drugs. We have previously reported the electrophysiological responses to convulsants using MEA in cultured hiPSC-derived neurons. In this study, we aimed to develop an analytical method enabling the evaluation of toxicity and the classification of MoA of convulsants using multivariate analysis and deep learning. hiPSC-derived cerebral cortical neurons were cultured on Micro-electrode array (MEA) plate, and the pharmacological responses over 10 drugs in spontaneous firings were obtained. We identified the parameter sets that can separate the responses between convulsive and negative control drugs, and the responses among the several convulsants with different action mechanism using principal component analysis. In principal component analysis and clustering method using the identified parameter set method will be effective for detecting convulsive response and predicting MoA of convulsive drugs. We also constructed the raster plots of spontaneous firing and the divided image data. The 4096 feature vectors of the divvied image data in raster plots were extracted by pre-trained model. Next, CNN model was trained with feature vectors each drug name. Using this trained CNN model, we have succeeded in separating the responses between non-convulsive drugs and convulsants, and classifying the MoA of convulsants. These multivariate analysis and deep learning methods are useful for the prediction of seizure liability and the classification of MoA of new drugs.

PS 2084 Altered AhR Signaling Disrupts the Expansion and Differentiation of Hematopoietic Stem and Progenitor Cells

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Hematopoietic stem cells (HSCs) give rise to all the cells of the blood and immune system. HSCs balance between self-renewal and differentiation in order to not only sustain homeostatic cell production, but also respond to stressors such as infection or injury. In response to environmental signals, HSCs proliferate and differentiate into multipotent progenitors (MPPs), with different MPP subtypes biased toward particular lineages. Yet, how environmental signals direct HSC transition to MPPs and influence lineage biasing are not fully understood. Growing evidence indicates that the aryl hydrocarbon receptor (AhR), an environment sensing transcription factor, regulates HSCs. For instance, HSCs from mice lacking AhR have an increased rate of proliferation. Additionally, mice lacking AhR have a greater proportion of mature myeloid cells. We hypothesize that the AhR regulates hematopoiesis by controlling HSC self-renewal, and also directs differentiation towards specific lineage-biased progenitors. We show that *in vivo* antagonism of AhR in mice using CH-223191 decreased the proportion of HSCs. Treatment with CH-223191 also increased the proportion of myeloid-biased MPPs (MPP3s). Using *ex vivo* colony forming unit (CFU) assays, *in vivo* treatment with CH-223191 promoted greater myeloid biased differentiation. Concomitant with HSC proliferation in response to stressors, *AhR* expression is downregulated. To further define the role of *AhR* downregulation, we utilized inducible global AhR knockout mice and found that they had a greater frequency of HSCs and MPP3s. In addition to signals from the hematopoietic compartment, hematopoiesis is regulated by bone marrow niche cells. To determine if the AhR affects HSCs and MPPs intrinsically or via signals from non-hematopoietic cells we utilized hematopoietic-specific conditional AhR knockout mice. Mice lacking AhR in the hematopoietic compartment had a greater frequency of HSCs and also a significant increase in myeloid biased progenitors. These findings indicate that the effects observed were due to changes intrinsic to the hematopoietic compartment. These findings implicate the AhR in the regulation of hematopoiesis by controlling HSC expansion and lineage biasing. This provides further insight into the factors that control steady state hematopoiesis and how hematopoietic stem and progenitor cells respond to environmental factors.

PS 2085 Deconvolution Algorithm Applied to RNA-seq Data Reveals Genetic Heterogeneity in the Differentiation of Induced Pluripotent Stem Cells (iPSC) Derived from Collaborative Cross Recombinant Inbred (CCRI) Mice into Fetal Liver

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Hepatocyte-like cells (iHeps) can be differentiated in culture from induced pluripotent stem cells (iPSCs) providing a useful tool for investigating a wide variety of liver associated pathologies. Since genetic differences in the population complicate the interpretation of toxicological study results we chose to use the Collaborative Cross (CC) mouse resource. Composed of 8 genetically extant founder lines that have been intercrossed for multiple generations to derive recombinant inbred (CCRI) mouse lines that breed true, CCRI iPSCs provide the opportunity to conduct genetically diverse studies *in vitro*. We previously showed that 3 independent CCRI iPSC lines differentiated to iHeps showed significant heterogeneity in phenotype and gene expression. Transcriptomes of these lines were analyzed at 3 time points during their differentiation using RNAseq with RT-PCR validation of selected genes. To further elucidate differentiation trajectories, we applied the multi-subject single cell deconvolution (MuSiC) algorithm and the mouse organogenesis single cell atlas (MOCA) to determine which cell types and trajectories were likely present in our cultures. For purposes of comparison, we focused on three cluster types in the MOCA data set: main cell types, main trajectories, and sub-trajectories, to estimate the proportions of each of these cluster types found in CCRI iHep bulk RNAseq data. Five main cell types were identified: stromal, erythroid, endothelial, and to a much lesser extent, hepatocytes. Trajectories recognized were haematopoiesis, endothelial, epithelial, hepatic, early developmental mesenchyme and glial neural crest. Although all three iPSC cell lines follow the same differentiation patterns there is variation among them especially in the haematopoiesis trajectory. These results suggest that this is a model for early fetal liver differentiation that includes blood forming elements naturally present in fetal liver. This CCRI iHep model system will be

useful for investigating the genetic bases of xenobiotic-induced fetal liver damage, including the genesis of blood disorders and potentially, childhood leukemia. *Supported by NIH P30ES007033, P30ES029067 and R01ES029911.*

2086 Development of a Murine Liquid Culture Bone Marrow Assay Using a High Content Analysis Platform

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Adverse hematological effects are frequently encountered and dose limiting for many drug therapies. The colony forming assay is valuable tool to assess and evaluate the hematopoietic adverse effects of compounds on bone marrow. The assay makes it possible to study both the proliferation and differentiation of hematopoietic stem cells and their progenitor cells however it is time and resource consuming. The murine CFU-GM assay has been validated to predict a drug candidate's potential toxicity, exposure levels & dose limitations in humans. The aim of the study was to develop a liquid culture based high throughput assay using the Thermo Scientific CellInsight CX7 High-Content Screening Platform to enable the study of both proliferation and differentiation. 17 compounds with in-house and/or published murine CFU-GM results were chosen for validation. Hydroxyurea, Didox, Busulfan, Bleomycin, Camptothecin, Topotecan, Zidovudine (AZT), Doxorubicin, Bortezomib, Paclitaxel (Taxol), 5-Fluorouracil, Melphalan, Etoposide, Cisplatin, Sunitinib, Flavopiridol and Clozapine. Characterization and optimization of the culture was further assessed using DAPI, F4/80, GR-1 (Ly-6G/Ly-6C), CD11b and/or C-kit. The assay was validated using the CX7 platform with a live/dead cell endpoint. IC50 values were obtained by using the CX7 spot detector protocol on the green (live) channel. IC50 values were calculated with GraphPad using a four parameter logistic regression model. Pearson correlation coefficient (r) and p values were calculated for all compounds. There is a strong positive (r values >0.8) correlation relationship between the published/in-house CFU assay and the liquid culture endpoint. The liquid culture assay is a suitable high throughput alternative to the resource consuming CFU assay in identifying bone marrow liabilities.

2087 Developing a Human Embryonic Stem Cell-Based High-Throughput Platform to Screen for Developmental Toxicants

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Every year, millions of infants worldwide are born with a serious birth defect, which raises the risk for lifelong disabilities to those who survive and increases the economic burden to their families and society. Besides genetic or hereditary factors, many of these defects can be caused by environmental chemical exposure, such as alcohol, smoking, and drugs. While there are over 80,000 chemicals registered for use in the United States, many of them have undergone little safety testing. Therefore, a rapid and accurate method for predicting developmental toxicants in the environment to humans is strongly desired. Pluripotent human embryonic stem cells (hESCs) possess the capacity to differentiate into any cell type which makes them an ideal *in vitro* model to investigate developmental toxicity. In this study, we aim to develop a transcriptomic-based high-throughput platform using hESCs to screen for environmental teratogens. Three-dimensional embryoid bodies (EBs) formed from hESCs were used as the model since its formation recapitulates many early embryogenic processes. 108 selected chemicals were first administered to EBs for 7 days to evaluate their toxicities. As the result, 64 impaired EB cell viability in a dose-dependent manner, 7 increased cell viability at high concentrations, while the remaining 37 showed no effect on EB formation within the tested concentration range. Next, the impacts of 23 chemicals on key embryogenesis signaling pathways and germ layer formation were investigated by measuring the expression of 37 hallmark genes of these processes. Hierarchical cluster analysis based on the transcriptional response change showed the ability to group chemicals with similar toxicity. For a more accurate categorization, a machine learning based prediction model was built. 10 chemicals with confirmed teratogenicity from TERIS (Teratogen Information System) were selected as the training controls. With recursive feature elimination, our model showed a high accuracy (mean: 0.82) and reliability (mean: 0.70) and the prediction results for the other 13 tested chemicals were similar with the findings from existing studies, with few discrepancies. Together, these results indicate that our screening platform could be successfully applied for identifying developmental toxicants and understanding their etiology.

2088 Capturing Drug-Induced Structural Changes in Images of Live Cells Using a Novel Deep Learning Method

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There has been significant progress in using cells differentiated from human induced pluripotent stem cells (hiPSCs) for modeling of human disease and for screening drug toxicity and efficacy. hiPSCs differentiated into specific lineages can represent human- and organ-specific properties for preclinical predictions of drug effects, including toxicity. To predict structural toxicity *in vitro*, cellular structures can be imaged using high-content microscopy, and image analysis algorithms used to generate quantitative measurements that relate to cell degradation or death, such as change in signal intensity or nuclear count. Such measurements do not typically capture subtle structural changes that are not easily visualized or are too complex to formulate with traditional image analysis and are rarely amenable to live-cell brightfield imaging. We have developed a novel method, PhenoTox, that uses deep learning for quantifying structural changes in cell-based models. The inputs are a collection of cellular images captured at multiple doses for the drugs of interest and a reference set of images with only the vehicle applied. Through the process of training 2-class deep neural networks, the system learns on its own what features within the images, if any, reflect structural changes. The output is a metric of structural change for each drug dose relative to the reference. Our technology introduces a new approach for structural toxicity testing that provides a high level of sensitivity that has not previously been possible. Our proposed method is agnostic to imaging modality and works for fluorescence images as well as bright-field images, enabling drug testing in live cell cultures. We present data from experiments using hepatocytes differentiated from hiPSCs in live cell cultures. Multiple doses of tamoxifen and doxorubicin (hepatotoxins) as well as aspirin and acetylcysteine (negative controls) were applied. Brightfield images were captured every 3 hours for 2 days using the IncuCyte imaging system. The time-lapse imaging data was analyzed with PhenoTox. Our method detected dose-dependent morphological changes caused by tamoxifen and doxorubicin over time and showed better sensitivity than a caspase-3/7 apoptosis assay. No significant structural change was detected for either aspirin or acetylcysteine.

2089 Human Induced Pluripotent Stem Cell Fluorescent Protein Reporter Lines for Dissecting Lineage-Specific Cellular Stress Response Dynamics Using High Content Imaging

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Cellular stress responses are critical for the repair of injured cells during diverse types of tissue damage, under pathological conditions as well as adverse responses to drug exposures. Targeting cellular stress response activation can contribute to cellular fitness and resilience to stress conditions. We established a reporter platform using fluorescent tagging of human induced pluripotent stem cells (hiPSCs) to visualize the dynamics of various cellular stress pathways on a single cell level. Both endogenous and scar free tagging was achieved using CRISPR/Cas9 technology, resulting in the maintenance of a representative cellular stress response *in vitro*. HiPSCs are an excellent model for *in vitro* safety screening since they rapidly divide, have undisrupted metabolic activity and can be differentiated into a whole range of cell types. This overcomes many of the limitations found in current *in vitro* models such as availability, inter-donor variability and stability. In order to dissect lineage specific oxidative stress response dynamics upon chemical exposure we differentiated oxidative stress hiPSC reporters, HMOX1-GFP and SRXN1-GFP, into different lineages including hepatocytes, cardiomyocytes, neuronal cells and kidney cells. The various differentiated cells were exposed to different oxidative stressors, CDDO-Me, diethyl maleate and paraquat followed by live cell imaging of the reporter activation using automated high content confocal imaging (HCI). We found large cell type dependent differences in amplitude of the oxidative stress response as well as the dynamic profile as a whole. This provides insights into organ specific sensitivity and dynamics towards chemically-induced oxidative stress response signaling. We anticipate that our hiPSC cellular stress response reporters in combination with HCI may play a key role for refined understanding of the dynamic stress response signaling in diverse target tissues under adverse drug reactions.

2090 Concentration-Dependent Toxicogenomic Changes of Silver Nanoparticles in Hepatocyte-Like Cells Derived from Human-Induced Pluripotent Stem Cells

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The widespread application of silver nanoparticles (AgNPs) has raised public concerns about their adverse effects on human health. However, information on their potential toxicity is still limited and sometimes controversial. Liver is one of the major organs where AgNPs accumulate after entering the blood stream. Therefore, *in vitro* models capable of accurately predicting hepatotoxicity of AgNPs are much needed. Primary human hepatocytes (PHHs) and hepatoma cell lines (HCLs) have mainly been employed in the past for *in vitro* hepatotoxicity assessment of AgNPs. The recent advent of hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (iPSCs) represents an attractive alternative *in vitro* liver model. Yet, the use of iPSC-derived HLCs for the study of nanoparticle toxicity has not been reported so far. In this study, we characterized transcriptomic changes induced by varying concentrations (5-25 µg/ml) of AgNPs in HLCs after 24 h exposure. AgNPs caused concentration-dependent gene expression changes in HLCs with increasing numbers of differentially expressed genes (DEGs) at higher concentrations. A total of 179 DEGs was identified at 5 µg/ml; the number increased to 936, 2859, and 4265 at 10, 20 and 25 µg/ml respectively. In all cases, members of the metallothionein (MT) and the heat shock protein (HSP) family were the dominating upregulated genes, suggesting that AgNP exposure induced cellular stresses and elicited cellular protective responses in HLCs. Functional analysis showed that the DEGs were majorly involved in the biological processes of metabolism, response to stress, and cell organization and biogenesis. Ingenuity Pathway Analysis revealed that cancer was at the top of diseases and disorders associated with the DEGs at all concentrations; hepatocellular carcinoma and liver hyperplasia/hyperproliferation were the leading hepatotoxicity responses. These results were in accordance with those reported previously on PHHs and HCLs. Considering the advantages HLCs have over PHHs and HCLs in terms of unlimited supply, consistency in quality, and sustainability of function in long-term culture, the results of the current study suggest that iPSC-derived HLCs may serve as a better *in vitro* model for liver nanotoxicology.

2091 Development of Assay-Ready iPSC-Derived CD34$^+$ Cells, Monocytes, and Mesenchymal Stem Cells

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There is an unmet need for highly characterized and reproducible cell models for a wide range of applications including mechanisms of action, drug development, and toxicity testing. Human induced pluripotent stem cells (iPSCs) have the potential to differentiate into all somatic cell types and therefore hold great promise for development into cell models for variety of toxicity applications. Herein, we have developed processes for the scalable generation of iPSC-derived CD34$^+$ cells, monocytes, and mesenchymal stem cells (MSCs). It is well documented that starting cell types and donor background play an important role in the efficiency of iPSCs to terminally differentiated cells. We screened four iPSC lines, three from bone marrow CD34$^+$ cells and one foreskin fibroblast-derived iPSC line, for differentiation into CD34$^+$, monocytes, or MSCs. All CD34$^+$ cell-derived iPSC lines exhibited high efficiency for differentiation into CD34$^+$ cells and MSCs, while the fibroblast-derived iPSC line favored differentiation into monocytes. To assess the differentiation potential of the iPSCs-derived cells, we demonstrated that CD34$^+$ cells could be differentiated into erythroid cells, myeloid cells, and megakaryocytes while monocytes were capable of differentiating into dendritic cells and functionally active macrophages. Compared to primary MSCs, iPSC-derived MSCs exhibited similar immunophenotypes and T-cell suppression properties as well as the ability to differentiate into adipocytes, osteoblasts, and chondrocytes. It can be problematic to obtain sufficient quantities of certain cell types, including CD34$^+$ cells, for high throughput drug screenings. To address cell availability and donor variation issues, we have developed processes for the scalable and reproducible generation of highly pure, functionally active, assay-ready iPSC-derived CD34$^+$ cells, monocytes, and MSCs. Furthermore, we have validated iPSC-derived monocytes for conducting the monocyte activation assay, which can be used as an alternative to the rabbit pyrogen test for biologic drugs. Utilization of CFU-GM assays for myelotoxicity screening is hampered by the low throughput of the assay. We have explored the potential of using iPSC-derived CD34$^+$ for *in vitro* assessment of anticancer drug-induced myelotoxicity. This assay further enhances the wide variety of applications. These highly characterized iPSC-derived cells provide as powerful tools for drug screening and toxicity testing.

2092 Effects of Electrical Stimulation on hiPSC-CMs Responses to Classic Ion Channel Blockers

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been widely used to assess the cardiac safety profiles of new drug candidates and hold great potential for personalized cardiac safety prediction, particularly for drug-induced proarrhythmia. However, hiPSC-CMs fire spontaneously, thus, the variable beating rates can be a confounding factor that interferes with data interpretation. Moreover, batch variations of hiPSC-CMs hamper their application for precision medicine. Controlling beating rates with electrical stimulation (E-pacing) or optogenetic pacing may reduce batch and assay variations, enable evaluation of rate-dependent drug effects, and facilitate the comparison of results obtained from hiPSC-CMs with results from adult human cardiomyocytes (CMs). As E-pacing of hiPSC-CMs has not yet been validated with high-throughput assays, herein, we compared the responses of hiPSC-CMs to the challenges of classic cardiac ion channel blockers under spontaneous beating and E-pacing conditions, utilizing the Axion Maestro Microelectrode Array (MEA). The rate-dependent drug effects were examined. Effects of E-pacing on different batches of hiPSC-CMs responses to I_{Kr} channel blockades were also evaluated. We found that compared to spontaneous beating hiPSC-CMs, E-pacing 1) reduced well-to-well, plate-to-plate, and batch-to-batch variabilities; 2) showed limited changes of FPD to I_f blocking; 3) revealed reverse rate-dependence of the responses to I_{Kr}, I_{Ks}, and I_{CaL} channel blockers in repolarization timing; and 4) eliminated effects of I_{Na} blocking on depolarization spike amplitude and spike slope due to the software defect. Optogenetic pacing of hiPSC-CMs showed use-dependent block of I_{Na} as found in adult CMs. In conclusion, pacing reduced batch and assay variations. Analyzing the responses of hiPSC-CMs in both spontaneous beating and pacing conditions may help better assess the effects of test compounds on cardiac electrophysiology and weigh the value of the hiPSC-CMs model for cardiac safety assessment.

2093 Simultaneous Assessment of Oncology Compound Efficacy and Toxicity in a Neuronal Human Induced Pluripotent Stem Cell and Glioblastoma-Based High-Throughput Screening Platform

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Glioblastoma Multiforme (GBM) are aggressive brain tumors with poor prognosis. Therapeutic management for GBM is currently limited; with Temozolomide currently being the standard of treatment for GBM. However, even when combined with radiotherapy only up to 20 percent of patients show favorable response to Temozolomide. Importantly, due to their requirements for potency and efficacy, oncology drugs often display appreciable toxicity profiles. In this study, the U87 GBM cell line was tagged with mCherry to serve as a traceable model for GBM. To study their growth behavior in a physiologically relevant system amenable to drug discovery, U87-mCherry cells were co-cultured with human induced pluripotent stem cell-derived cortical neurospheroids in a high throughput based screening platform. The neurospheroids are composed of a balanced co-culture of cortical neurons and astrocytes with mature functional neuronal circuitry evidenced by spontaneous calcium oscillations. Previously, this neurospheroid model has been successfully utilized in independent neurotoxicity and drug discovery studies. Thus, by combining U87-mCherry and the high throughput neurospheroids, the proliferation and infiltration patterns of the GBM cells as well as the preservation of cortical functionality can be analyzed within the same high throughput platform. Proof-of-principle studies focused on Temozolomide, Carmustin, Paclitaxel and Cisplatin to identify and profile compound efficacy versus their toxicity with the ultimate goal of providing a platform that can separate safe and effective compounds from those that have minimal efficacy and deleterious toxicity. Interestingly, Cisplatin seemed able to impact the proliferation and survival of the GBM cells, however it did cause detectable toxicity seen as impact in neuronal functionality. Tomozolomide did not cause neurotoxic effects, however it also did not impact the proliferative and invasive behavior of the GBM cells. The effects of Carmustine and Paclitaxel were unremarkable at the concentration ranges tested in this study. In summary, the work described herein suggests a novel framework approach for combining human induced pluripotent stem cell-derived cortical neurospheres with GBM, enabling more streamlined drug discovery of compounds against aggressive brain cancers.

2094 Validation of a qPCR Assay for Human Cell Biodistribution Assessment in Rat Tissues

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Cell therapies today hold enormous promise for treating many diseases. Determination of the fate of the human cells following administration in a nonclinical species can contribute to the assessment of its safety profile for investigational cell therapies. This can be achieved using quantitative polymerase chain reaction (qPCR). Successful qPCR assay validation requires careful scientific support as no regulatory guidance exists for the analytical validation for this assay type. We validated a qPCR assay based on specific detection of the human AluY repeat sequence corresponding to primate-specific short interspersed elements that represents 6-13% of the haploid genome. A fit-for-purpose assay validation approach was thus used, taking into account the validation parameters in the bioanalytical method validation guideline. Assuming that a human cell contains 7.0 pg of DNA, the calibration curve ranged from 1 to 2857 human genomes per µg of rat DNA, corresponding to ~1.5 $\times 10^5$ rat genomes. The maximal CV% values of the within- and between-run precision were 9.9 and 7.7%, respectively. The qPCR assay displayed high accuracy with maximal RE% of 14.8%, and a very low limit of detection (6.5 x 10^{-3} human genomes per µg of rat DNA). The qPCR assay was linear, with a range from 0.02 to 1 µg of rat DNA. Assay selectivity, specificity and matrix effect were evaluated in a series of qPCR experiments on rodent genomic DNA spiked or not with known amounts of human reference genomic DNA. The qPCR detection assay was found to be selective and specific in DNA from rat tissue and without a significant matrix effect. DNA extraction yield and stability in homogenates from rodent tissues were also evaluated. The DNA extraction process was considered as acceptable, except for blood and testes (sub-optimal yields). Cell stability in blood and tissue homogenates was demonstrated for up to 27 weeks at -80°C. This fit-for-purpose assay validation demonstrated that this qPCR assay displays adequate performance and a high sensitivity for quantitation of human cells in rodent tissues within the framework of a cell therapy biodistribution study.

2095 Statistical Modeling for Predicting Subacute Hepatotoxicity Induced by Distinct Surface Charged ZnO Nanoparticles in a Swiss Albino Murine Model

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Nanoparticles (NPs) interacts with biological molecules such as proteins, cell membranes, and nuclear material in a multi-pronged interfaces dictating their biological outcomes. This NPs/biological interfaces predominantly depends on colloidal forces as well as dynamic physico-chemical properties of NPs. These interactions were governed by size, solubility, shape, surface area, and surface chemistry of the NPs. In present study, we examined the role of surface charge to elicit the charge dependent hepatotoxicity of commercial zinc oxide NPs (ZnO) in murine model, Swiss Albino mice to probe the mechanistic role of surface charges of NPs on its toxicity outcome. Three specific ZnO NPs,neutral, positive and negatively charged particles were prepared by suitable coating with natural polymers (guar gum, chitosan and alginate respectively). These engineered ZnO NPs were characterized by advance spectroscopic and microscopic tools. NPs ionization study was performed in Milli Q, neutral PBS, gastric pH, lysosomal pH, and FBS to assess the impact of medium on degree ionization. The induced hepatotoxicity and bio-availability of different surface coated ZnO NPs have been tested in the mice model at dose level of 10, 50 and 300 mg/kg after sub-acute oral treatment. The dose, surface charges and time dependent hepatotoxicity of NPs based on biochemical parameters (AST and ALT) were evaluated by using multivariate optimizing Response Surface Methodology (RSM). ANOVA indicated appropriateness of the model for predication of hepatotoxicity owing to "Prob. >F" less than 0.05 for variable parameters. At RSM predicted optimal condition, positive charged NPs at the dose of 300 mg/kg showed higher toxicity than other groups. RSM models were validated using the method of cross-validation. Additionally the induction of ROS and histopathological lesions in liver were also support the RSM prediction.

2096 Inhalation Exposure to Cellulose Nanocrystals: Study of Pulmonary and Reproductive Outcomes in Male Mice

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Cellulose(s) are bio-based lightweight natural materials with high aspect ratios, excellent physical strength, transparency and chemical resistance. Crystalline nanocelluloses (CNC) have better electrical, optical, and mechanical properties compared to non-nanosized original forms. These features are very desirable for a number of novel applications. Eco-friendly technology and sustainability along with biodegradable nature made CNCs very attractive for manufacturing and high demand in industrial world market. We investigated adverse pulmonary and reproductive outcome caused by inhalation exposure to CNC aerosol generated from a bulk supply of wood pulp derived cellulose nanocrystals (freeze dried powder, FPL, USFS). C57BL6/J male mice were exposed to precise concentration of dispersed airborne CNC (5 mg/m^3, 5 h/day, 5 days/week for 3 weeks). Measurements of pulmonary functions in mice were conducted prior to bronchoalveolar lavage (BAL), lungs and testes collection for histopathology, and measurements of oxidative stress, inflammation, and effects on male reproductive functions at 24 h, 2-, 6- and 12-months post exposure. Exposure to CNC significantly increased airway responsiveness to methacholine and decreased tidal volume at 12 months of recovery as compared to air-control group. Hierarchical clustering analysis of the inflammatory cytokine responses revealed a shift in inflammatory responses from a Th1 type at early recovery time points to a Th2 response at the later stages. Similarly, BAL cytology indicated the preferential accumulation of polymorphonuclear neutrophils (PMNs) or eosinophils, respectively, at 24h and 12 months post exposure. Moreover, CNC inhalation resulted in the prominent perivascular lymphoid aggregates along with stronger peribronchial and perivascular fibrosis in the lungs (6-12 months of recovery). Histological analysis of testes showed pathological manifestations suggestive of abnormal sperm functions and production at the later time points of recovery. Inhalation exposure was associated with sperm DNA damage, changes in sperm motility and morphology. Accumulation of oxidative damage was observed as evidenced by elevated contents of oxidatively modified protein carbonyls in the testes. In conclusion: Exposure of C57BL6 mice to respirable CNC caused pulmonary and male reproductive toxicity observed up to 12 months post inhalation.

2097 Molecular-Level Insight into Adverse Outcome Pathway for Complex Metal Oxide Nanomaterial Exposure Using *Chironomus riparius*

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Lithium cobalt oxide (LCO) nanomaterials, contained in the cathode material of lithium ion batteries (LIBs), comprise a growing category of industrial production and commercial waste, with LIB waste from electric vehicles alone expected to reach 200 kilotons annually by 2025. A mechanistic understanding of the toxicology of this material will be important for understanding the implications of exposure to this material, including for the environment. In aqueous media, this material can aggregate and settle, concentrating at the bottom of the water column, which would create particularly high exposures for benthic organisms. A model for such exposure is the midge larvae *Chironomus riparius*. Exposure of *C. riparius* larvae to LCO concentrations as low as 10 mg/L cause reductions in size, lower levels of hemoglobin, and delays in development. Molecular studies by qPCR, RNA-Seq, enzyme assay, and electron paramagnetic resonance reveal negative impacts of LCO on Fe-S centers of proteins important for metabolism and regulation of heme production. These findings inform an Adverse Outcome Pathway for LCO exposure, whereby LCO impacts on protein Fe-S centers cause metabolic impairments that result in stunted growth and delayed development.

2098 Sex Differences in Acute and Chronic Lung Inflammatory Responses to Nickel Nanoparticles

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Nickel nanoparticles (NiNPs) are widely used in various technological applications. A strong association between nickel inhalation exposure and asthma, pulmonary fibrosis, and lung cancer has been established in humans. In general, epidemiology studies show that women are more susceptible than men to chronic lung inflammation, while men are more susceptible to acute inflammation. Therefore, we hypothesized that male mice would be more susceptible to acute lung inflammation, whereas female mice would be more susceptible to chronic lung inflammation when exposed to LPS or/and NiNPs. The goal of this study is to explore mechanisms of susceptibility between male and female mice to acute and chronic lung inflammation after NiNP exposure. Methods**:** For the acute study, C57BL/6J male and female mice were treated by oropharyngeal aspiration with vehicle (0.1% Pluronic in PBS), LPS (5 µg/kg), NiNPs (4 mg/kg), or both, and sacrificed 24 hrs post-exposure. For the chronic study, mice were exposed to vehicle, LPS (0.83 µg/kg), NiNPs (0.67 mg/kg), or both at day 1, 3, 5, 15, 17, and 19, and sacrificed 24 days post initial exposure to the stimulants. Bronchoalveolar lavage fluid (BALF) was collected from the lungs for analysis of inflammatory markers. The lungs and other organs were collected for RNA and protein analysis or histopathology. Results: The acute study showed that male mice had higher IL-6 and neutrophils in their BALF after NiNP or LPS/NiNP treatment. Although total STAT3 levels measured by Western blot were similar, NiNP-induced STAT3 phosphorylation was significantly higher in male mice. This could be due to the inhibitory action of estradiol on IL-6 production, the main activator of STAT3. For the chronic study, we observed increased numbers of monocytes in the BALF of male mice after NiNP or LPS/NiNP exposure. Male mice also had higher levels of CCL2 in their lung lysates compared to the female mice. We suggest that the mechanism of male susceptibility to acute NiNP-induced lung inflammation involves increased neutrophilic infiltration regulated by IL-6/STAT3, whereas male susceptibility to chronic NiNP-induced lung inflammation involves monocytic infiltration regulated by CCL2. Overall, these findings suggest that human acute or chronic exposure to NiNPs and/or LPS would result in more severe lung inflammation or the inefficient resolution of inflammation in males. *Funding: Supported by NIEHS grant R01-ES020897 and NIEHS grant T32-ES007046.*

2099 Potential Toxicity of Graphene Oxide Nanomaterial to Japanese Medaka Fish

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Due to unique physico-chemical properties, graphene oxide (GO) has shown great potential for biomedical, energy and electronic applications. As a result, release of GO into the ecosystem especially into the aquatic environment is inevitable; however, the potential risks on aquatic life are almost unknown. We have evaluated the toxic potential of GO on Japanese medaka fish (*Oryzias latipes*) adults. Reproductively active male and female medaka fish were intraperitoneally injected with GO (25-200 µg/g; single injection). The exposed fish were paired and allowed normal breeding for three weeks. The laid eggs were evaluated for hatching, fertility and developmental abnormalities. Males were more sensitive to GO toxicity than females; the calculated EC_{50} values were 172.9 µg/g and 1766 µg/g for males and females, respectively. Histological evaluation of the gonads (testis and ovary), liver and kidney of fish three weeks post injection showed that GO agglomerated in the peritoneum and accumulated in different organs including ovary, even though the fish were able to maintain normal breeding. The embryos were able to maintain normal development with occasional cardiac and yolk edema. GO accumulation produced organ damage including fibrosis of the ovary and interstitial cell hyperplasia of the kidney (especially in males). Taken together, our data indicate that GO is toxic to Japanese medaka fish, and the males being more sensitive than females. GO also accumulated in different organs and produced an oxidative damage that may initiate aggregation of macrophages in the damaged sites leading to necrosis and/or granulomatous inflammation. *Supported by NIMHD grant # G12MD007581and NSF grant #HRD 1547754.*

2100 Chronic Exposure to the Food Additive Silicon Dioxide (E551) at a Human-Relevant Dose Blocks Oral Tolerance Induction and Induce Food Intolerance in Mice

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Food-grade SiO_2 (E551 in EU), composed of aggregated nanoparticles (NPs), is used as an anticaking and antifoaming agent in powdered foods, with chronic dietary exposure in humans (0.8-74 mg/kg/day). In mice, SiO_2-NP models block induction of oral tolerance (OT) to dietary antigens. The current study in mice aimed at evaluating the effect of chronic exposure to E551 at a human-relevant dose, added to a solid food matrix or in liquid suspension, on OT induction to the food antigen model ovalbumin (OVA). Mice were daily treated for 60 days without (controls) or with E551 (10mg/kg/day) in water suspension (gastric gavage) or incorporated into food pellets (solid matrix). Food intake was controlled. At day 41, OVA (20mg/mouse; OVA-tolerized mice) or PBS vehicle was orally administered for 3 days in each group. All mice were subsequently immunized by subcutaneous injection of OVA (100µg/mouse) on day 48. Blood was collected 1 week after for anti-OVA IgG serum titers to evaluate OT induction in OVA-tolerized mice exposed or not to E551. In all groups, to further assess OT to food antigens, mice were orally challenged by OVA (25µg/mouse) for 5 days before sacrifice. Isolated immune cells from mesenteric lymph nodes (MLN) were activated by PMA/ionomycin to assess pro- (IFNγ) and anti-inflammatory (IL-10, TGFβ) cytokine secretion by ELISA. Fecal lipocalin (Lcn)-2 level was used as a global marker of gut inflammation. In control mice, OVA tolerance protocol (oral OVA) lowered by 87% circulating anti-OVA IgG levels ($p<0.0001$) compared to oral PBS group, showing OT induction to OVA. In contrast, anti-OVA IgG titers did not decrease in OVA-tolerized mice chronically exposed to E551 regardless of the vehicle, showing blockade of OT to food antigens. In OVA-tolerized mice exposed to E551 through food pellets, *de novo* oral challenge with OVA led to an increase ($p<0.05$) of fecal Lcn-2 levels (+131%) and IFNγ (+139%) production by MLN cells, together with a drop ($p<0.05$) of TGFβ (-46%) and IL10 (-46%) when compared to OVA-tolerized controls, demonstrating gut inflammation. These results showed that chronic E551 exposure at human dietary levels in solid or liquid matrix impairs OT to dietary antigens, and promotes intestinal inflammation supporting food intolerance.

2101 Silver Nanoparticle-Induced Differential Biodistribution and Inflammatory Responses in Healthy and Obese Models

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Silver nanoparticles (AgNPs) are proposed as an antibacterial agent in many biomedical applications, including surgical devices and implants. While research regarding their toxicity has primarily been performed in healthy models, the evaluation of AgNP toxicity in diseased conditions is of critical importance, as a substantial and growing proportion of the population suffers from a pre-existing condition. Specifically, approximately 40% of adults and 20% of children in the United States are obese. Our current evaluation utilized both healthy and obese mouse models to determine potential variations in AgNP toxicity. We hypothesized that obesity would alter inflammatory responses due to differential AgNP biodistribution. Healthy and obese C57BL6 mice were injected via tail vein with 2mg/kg AgNPs. After 24 h, the organs were collected and assessed for Ag content by atomic absorption spectroscopy and expression of inflammatory genes including IL-6, TNF-α, CXCL1, CXCL2, CCL2, TGF-β, IL-1β, HO-1 and others via real-time rtPCR. AgNPs induced differential total and relative inflammatory responses in healthy and obese mice. While AgNP exposure consistently caused an increase in the expression of inflammatory genes (IL-6, TNF-α, CXCL1, CCL2, TGF-β, IL-1β, HO-1) within the spleen of healthy mice, obese mice demonstrated decreased gene expression of CXCL1, CCL2, TGF-β, and IL-1β. Both healthy and obese mice exhibited similar inflammatory responses to one another within the liver. AgNPs localized primarily to the liver and the spleen, though Ag was present in all organs that were tested, including the brain, kidney, aorta, intestines, heart, lung, and serum. Biodistribution differences were observed between models. More Ag was deposited within the liver, kidney, heart, and serum of the obese mice compared to healthy. For the intestines and lungs, more Ag was detected in the healthy than the obese. The aorta, spleen, and brain contained the same amount of Ag between groups. Induction of inflammatory genes was not determined to correspond with biodistribution, suggesting disease-related variations in AgNP-induced toxicological responses. Together, these findings indicate that prevalent underlying diseases may impact the clinical application of NPs by influencing biodistribution and immune responses.

2102 Oral-Administered TiO_2 Nanoparticles Impact Basic Neurobehavioral and Cardiac Assessments and Biochemical Profiles in Rat Pups

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Titanium dioxide (TiO_2) is one of the most prevalent nanoparticles (NP) used as a food additive, and is found in baby formula, baked goods, candy, dairy products, etc., making ingestion unavoidable. Little is known about biological responses to NP exposure during infancy when both the intestinal tract and brain is still undergoing considerable development. In the study presented here male and female rat pups received four oral doses of 10 mg/kg TiO_2 P25 NP between postnatal day (PND) 2-5, 7-10, or 17-20, and were sacrificed at PND 21. Basic neurobehavioral (acoustic startle response, locomotor activity, and rotarod) assessments were performed at PND 20. Cardiac assessment (ECGenie) was conducted on PND 14 and 20. Six neurotransmitters/metabolites were quantified in brain by UPLC with electrochemical detection. Plasma metabolites (n = 181) were quantified using AbsoluteIDQ® p180Kits (Biocrates) analyzed by LC-MS. TiO_2 administered between PND 17-20 caused a decrease in locomotor activity ($P<0.05$). Dosing between PND 7-10 resulted in a decrease in heart rate in both male and female pups, however when dosed between PND 17-20 only female pups had a significantly decreased heart rate. Neurotransmitters/metabolites analyses show dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and norepinephrine (NE) were impacted in an age- and gender-specific manner: DA was increased for male and female pups dosed between PND 2-5 but decreased for males dosed PND 17-20. DOPAC was increased for male and female when dosed PND 7-10. HVA were decreased following administration at PND 7-10 and 17-20. NE were decreased for females dosed PND 17-20. Metabolomic analysis of plasma shows between 6-17 individual metabolites and metabolite ratios/sums were significantly different between exposure groups and vehicle controls. Our results show that the biological responses to orally dosed TiO_2 NP in early life happen in an age- and gender-specific manner. Our data suggest that TiO_2 NP may impact neurobehavioral and cardiac performance, neurotransmitter/metabolite concentrations in brain, as well as metabolite concentrations in plasma.

2103 Understanding the Variable Drivers of Toxicity for the Broad Class of Carbon Nanotubes and Nanofibers from US Facilities

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Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F) is known to induce inflammation, toxicity, or tumorigenesis, and is a concern in the occupational setting. Previously, we established toxicity profiles from male C57BL6/J mice aged 8-10 weeks exposed to either 4 or 40 µg of one of nine different CNT/F via oropharyngeal aspiration as well as human epithelial BEAS-2B cells (0-24 µg/ml), differentiated THP-1 cells (0-120 µg/ml), and human fibroblasts (0-2 µg/ml) for four primary outcomes of genotoxicity, inflammation, pathology, and translocation. An overarching goal of our expansive study was to use machine learning to determine the relationship between particle physicochemical characteristics with respective toxicity outcomes and the relationship between those four primary outcomes. The nine materials had a wide range of characteristics including diameter (6-397 nm), length (0.1-50 µm), surface area (18-238 m^2/g), aspect ratio (2-1396), residual metal catalyst (0.3-6.2 %), density (0.007-0.220 g/cm^3), etc., to consider. Unsupervised approaches were used to identify and define subsets of materials with similar outcomes. Subsequently, supervised learning approaches were used to identify physicochemical characteristics that define these outcomes. While some physicochemical characteristics were determined to be key drivers of specific toxicity outcomes, different characteristics were essential when considering other toxicity endpoints. More specifically, drivers of inflammation and/or pathology were not the factors driving translocation and/or genotoxicity. No single characteristic could be used as a toxicity predictor, therefore, multifactorial processes, or combination of characteristics, were necessary for an accurate and effective prediction model for responses. The study identified physicochemical drivers of CNT/F toxicity using an integrated approach, combining experimental evidence with computational modeling, with potential for broad application.

2104 A Long-Term Whole-Body Inhalation Study of Multiwalled Carbon Nanotubes in Mice with an Improved Dispersion and Inhalation System

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Inhalation study is essential for assessment of general respiratory toxicity including non-cancerous endpoint of the nanomaterials (NMs). In designing inhalation studies for NMs, it is important to control the status of aggregate and agglomerates (AA) to be consistent with the human exposure. For example, a multi-wall carbon nanotube (MWCNT) such as Mitsui MWNT-7 is a mixture of single fibers (SF) and their AA. Human ambient air, in general, is less agitated; the AA may sediment away faster, SF may suspend longer in the air and to be inhaled by humans. On the other hand, the air for animal experiments is rigorously agitated in order to ensure the homogeneity of the aerosol; when the aerosol is a mixture of SF and AA, the AA could be trapped by the upper respiratory tract and impede the SF to reach the alveolar region consequently induce AA-specific lesions (e.g. granulomas) which are considered to block and/or mask the nature of toxicity induced by the SF. Taking all into account, we considered that it is essential to make a well-dispersed aerosol without AA for the human-relevant exposure scenario. To generate well-dispersed aerosol, we developed the "Taquann" dispersion method and a "direct injection" whole body inhalation system for the dispersed sample (designated as "Taquann System" J Toxicol Sci. 2013). In this study, we have conducted a long-term inhalation exposure study of MWCNT (MWNT-7, Mitsui) by using Taquann system. Male C57BL/6 mice, 12-week-old were exposed to MWCNT aerosol, 2.7 and 5.1 mg/m^3 in the low concentration group (LC) and in the high concentration group (HC), respectively. Duration of exposure was 6 hrs a day for 6 months every 4 weeks. MMAD on aerosol in the chamber was approx. 500 nm measured by MOUDI (Model125, Kanomax). Lung burden of the first time exposure were approx. 4 and 6 microgram per animal in the LC and in the HC, respectively. The fiber length recovered from the lungs was approx. 6.5 micrometer. Histologically, SF were found around terminal bronchioles to alveolar region. Macrophages surrounded and/or phagocytized cluster of SF. However, clear formations of granuloma were not observed. It is concluded that aerosol dispersibility should be considerations for risk assessment of NMs. *This work was supported by the Health and Labour Sciences Research Grant, Japan.*

2105 The Effect of Disease State and Age on the Biodistribution Profile of Injected Gold Nanoparticles

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Many nanoparticle (NP) biodistribution studies focus on characteristics such as size, shape or dissolution that affect tissue accumulation and retention in healthy animals. However, little has been done to determine how factors such as disease state or age can alter barrier permeability and ultimately change NP biodistribution. Neuroinflammatory diseases, such as Alzheimer's disease (AD), often feature a compromised blood-brain barrier (BBB). Thus, we hypothesized that AD state and age would alter the biodistribution of blood-borne NPs, particularly to the brain. To study the effects of AD- and age-related changes in tissue NP accumulation, we used the 3xTgAD mouse model and varied age as a function of disease state. Young (3 mo) and aged (19 mo, 23 mo) 3xTgAD and non-transgenic (NTg) mice were injected intravenously with colloidal gold NPs (AuNPs, primary particle size 20 nm, 45-49 µg per mouse). Tissues were collected 24 hrs post-injection and processed by acid-assisted microwave digestion. Au tissue content was determined by atomic absorption spectrometry (liver, spleen) or inductively-coupled plasma mass spectrometry (kidneys, blood, microdissected brain). The total recovered Au dose in all tissues measured was higher in the aged mice as compared to young mice irrespective of genotype ($p=0.0012$). The biodistribution profile for both genotypes demonstrated that the livers accumulated the highest relative dose (65-85% of the recovered dose), followed by the spleen (14-33%), kidneys (2-3%), blood (<1%), then whole brain (<0.5%). Analysis of Au tissue concentrations in microdissected brain regions (olfactory bulb, hippocampus, striatum, frontal cortex, cerebellum) demonstrate regional differences in brain accumulation by age and genotype. When considering the brain as a whole, there was a significant interaction effect of age and genotype on Au accumulation ($p=0.006$), where elevated levels of Au were found with in-

creased disease progression. Levels of Au in blood filtering organs such as the liver, spleen, and kidneys demonstrated either no effect or decreased levels of Au with age. Overall, these findings suggest that the BBB is comparatively more permeable to blood-borne NP accumulation due to AD- and age-related changes as the liver, kidneys, and spleen did not show these same effects. *Funding Sources: ExxonMobil Biomedical Sciences, R01ES020332, T32ES007026, P30ES001247.*

2106 Assessment of Chronic Inhalation Exposure of Thin and Long Multiwalled Carbon Nanotubes in Rats and Mice

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Multiwalled carbon nanotubes (MWCNTs) are a diverse class of nanoscale materials with a broad range of industrial uses. While one type of MWCNTs with physical attributes of a nanoscale fiber has been classified as probably carcinogenic, MWCNTs as a class are currently unclassifiable regards their carcinogenic hazard due to a lack of available data. A relatively thin and long MWCNT (2.6 um long x 15 nm wide) with high purity (>99%, residual nickel present at 0.52%) was selected as a test material for long-term whole-body inhalation toxicology and carcinogenicity studies. Consistent with reports of MWCNT observed in occupational settings, this material existed as micrometer scale agglomerates in the bulk test material and also in the inhalation exposure atmospheres. Using this material, 30-day and 2-year toxicology and tissue burden studies were conducted in male and female Sprague Dawley (HSD: Sprague Dawley® SD®) rats and B6C3F1/N mice. In the 30-day studies, the higher exposure concentrations (3 and 10 mg/m^3) were associated with reduced pulmonary clearance, inflammation and hyperplasia. To aid in the design of a chronic study, the lung burden data were modeled to predict the exposure concentration that would result in pulmonary overload at 18 months of exposure at the highest exposure concentration. Based on this information, rats and mice were exposed to 0, 0.06, 0.2 or 0.6 mg/m^3 (6 hr/day, 5 days/week) by whole body inhalation for up to two years. In the 30-day study there was negligible pulmonary toxicity at the range of these concentrations selected for the chronic study. In the chronic study, at the 12 month interim assessment, in general there was no effect of exposure on terminal body weights. There were modest but significant increases in relative lung weights in both male and female rats, and male and female mice. Lung burdens of MWCNT increased with exposure concentration and duration. The increase in lung burdens was consistent with the exposure concentration dependent increase in severity of "foreign material" diagnosed in the lung of exposed rats. Increases were greater than proportional as exposure concentration increased indicating that MWCNT were not effectively being cleared from the lung at the higher concentrations and as a result did not reach steady state in either species.

2107 Hepatotoxicity of OH-*f* MWCNTs in Wistar Rats

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Carbon in nano-form is a superstar material for 21st century nano-scientists. Multiwalled carbon nanotubes (MWCNTs) have potential applications in the field of electronics, textiles, water purification, nanocomposites and biomedical applications such as targeted drug delivery, biosensors, tissue engineering among others. Toxicity and biocompatibility studies have become very important before these materials enter our daily life in a big way. In the present study, OH-functionalized MWCNTs (OH-*f* MWCNTs) were homogeneously suspended in autoclaved normal saline with 0.1% Tween-80 by probe sonication method. Adult Wistar rats were divided into four groups namely control group, low-dose group, mid-dose group and high-dose group (each group having 6 animals) and each group was administered intraperitoneal doses of 0.0 mg/kg BW, 0.4 mg/kg BW, 2.0 mg/kg BW and 10.0 mg/kg BW respectively. Autopsy was done after 15 alternate doses over a period of 30 days. Biochemical assays for alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and oxidative stress assays (catalase and malondialdehyde) were performed. Liver histopathology was carried out in hematoxylin-eosin stained sections. A dose-dependent hepatotoxicity pattern was observed post OH-*f* MWCNTs exposure. Feeding pattern, body weight and organ indices did not change significantly during the exposure period. Concentrations of ALP, AST and ALT were altered significantly in the sera of mid and high-dose treated rats. Catalase enzyme activity in liver tissue homogenates was lowered in mid-dose and high-dose group. Malondialdehyde levels increased in animals treated with 2.0 mg/kg BW and 10.0 mg/kg BW. Histological analysis of hepatic tissue revealed dose-dependent pathological changes in structure. Mid and high-dose groups exhibited several necrotic areas especially near the central vein. Inflammation was also observed around the central vein and portal veins. Many hepatocytes showed abnormal features such as degenerated nucleus, vacuolation, cell shape changes and cell swelling. These findings indicate that multiwalled carbon nanotubes damage the structure of hepatic tissue and impair function as well. Further studies are required to understand the mechanistic aspect of hepatotoxicity caused by MWCNTs.

2108 Acute Pulmonary Toxicity of Aerosolized Zinc Oxide Engineered Nanomaterials in Rats

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There are more than 1600 engineered nanomaterial (ENM)-based consumer products on the global market today with annual sales of $70 million in the US alone. Zinc Oxide (ZnO) nanoparticles are commercially used as antibacterial, antifungal, anti-corrosive and UV filtering agents. Increased consumer product use has created concerns for the potential of increased risk by inhalation of these nanoparticles during synthesis and commercial application. ZnO nanoparticles have been reported to cause toxic effects by dermal, ingestion and inhalation routes of exposure. The primary objective of this study is to determine if inhalation of ZnO nanoparticles induces inflammation in the lungs. Male Sprague Dawley rats were randomly assigned to sham control (n=19) or ZnO inhalation (n=26) groups. Rats were exposed to aerosolized 50 nm diameter ZnO (1mg/ml) for a single 6 hour period and examined at 0, 1, 7 and 21 days following inhalation. Bronchoalveolar lavage (BAL) was collected from the right lung to assess protein concentration, cell number, viability, and cell differentials. The left lung was inflation-fixed, embedded and sectioned for histopathological analysis. Particle aerosol was characterized by gravimetric measurement, x-ray fluorescence (XRF), cascade impactor and transmission electron microscopy (TEM). The generated ZnO aerosol mass was 4.23 +/- 1.27 mg/m^3. The concentration of Zn in the aerosol by XRF analysis was 2.5 +/- 0.2 mg/m^3. Mass median aerodynamic diameter of the aerosol was 3.57 µm, while the average median size of nanoparticles measured by TEM was 118 nm. TEM demonstrated limited agglomeration of ZnO nanoparticles following aerosolization ranging from 50 to 340 nm in size. Day 0, 1 and 7 post-exposure (PE) demonstrated a significant decrease in the percent of viable cells in BAL following ZnO exposure ($p<0.05$). Macrophages were significantly increased on day 0 PE compared to controls. Neutrophils were significantly higher following ZnO inhalation on day 0 and 1 PE ($p<0.05$). BAL protein on day 7 was higher compared to control. Histopathological lung assessment showed increased numbers of macrophages in the airways and pleural regions positively stained for HO-1, a marker of oxidative stress. These findings demonstrate ZnO can lead to short-term inflammation and decreased cell viability, suggesting ZnO nanoparticles are not completely innocuous when inhaled.

2109 Investigating the Toxicology of Intramuscular Injected CNT-AB in Mice Followed by Microwave Hyperthermia

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The advent of carbon nanotubes (CNT's) has prompted a wide range of research in various fields but more specifically into cancer therapy for localized and site-specific treatment. Carbon nanotubes bound to tumor-specific antibodies (Ab) offer specific treatment for cancer cells without affecting the surrounding tissue. It is hypothesized that this treatment will make use of the infrared absorptive properties of nanotubes and will produce systemic effects that are indicative of abolished cancer cells. We seek to affirm the initial results of CNT in cancer therapy by investigating the toxicology in mice injected with CNT-Ab followed by microwave hypothermia. The toxicological results from the animal models will help guide the development of this treatment and potentially validate the method as a viable and effective form of cancer treatment for humans. After 2 weeks, blood was collected from the mice to analyze albumin, total protein, aspartate transferase (AST), and creatinine in blood serum. At the end of treatment, mice were sacrificed for examination of gene expression in liver, pancreatic, and brain tissues. Total protein concentration was varied across treatment groups. There were no significant changes in albumin levels as compared to the control group. The 0.125 mg/ml anti-PSMA-MWCNT (No Microwave) and 0.5 mg/ml anti-PSMA-MWCNT + Microwave treatment groups showed reduced total protein while the Microwave only and 0.125 mg/ml anti-PSMA-MWCNT + Microwave treatment groups showed elevated total protein levels compared to the control. With respect to AST results, Microwave only, 0.125 mg/ml anti-PSMA-MWCNT + Microwave, and 0.5 mg/ml plain MWCNT + Microwave treatment groups showed elevated

levels while the 0.125 mg/ml plain MWCNT + Microwave treatment group showed reduced levels. The majority of groups given microwave treatment had lower levels of creatinine. All treatment groups showed an upregulation of NFKB and TNF, while IL6 and PTSG2 were downregulated and IL1B was not expressed at all. Results showed minimal to no effects two weeks after a single injection in mice with CNT-Ab followed by microwave hypothermia. Study is still ongoing to examine histopathological data.

2110 A Toxicological Assessment of Crystalline Nano-Cellulose in Mice

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Crystalline nanocellulose (CNC) is derived from the natural polymer, cellulose. Considering the numerous applications of CNC, a substantial number of workers could be exposed to CNC. This study investigates the time-course of onset, progression, and resolution, if any, of CNC-induced toxicity. For hazard ranking, the outcome of the CNC toxicity studies was compared to those induced by a larger-sized crystalline micro cellulose; CMC, and to a reference material, Mitsui multi-walled carbon nanotubes (MWCNT). Mice (male, C57BL/6J, 7 weeks old) were administered dispersion medium (DM) or DM containing CNC, CMC (50, 100, and 200 µg/mouse), or MWCNT (50 µg/mouse) as a single pharyngeal aspiration. At 1 day, 7 days, 1 month, 3 months, and 6 months post-exposure toxicity facilitated by exposure to the varying materials was assessed. Serum transaminases were measured to determine hepatotoxicity. Whole lung lavage was conducted to assess pulmonary toxicity (PMN number and LDH activity). Sperm counts and motility analyses were used to assess reproductive toxicity. All doses of CNC caused a significant increase in PMN number and LDH activity at 1- and 7-days post-exposure. For CMC, an increase in PMN number and LDH activity was also found at 1-day post-exposure, for all doses. However, the inflammatory response of CNC (number of infiltrating PMNs) was significantly greater when compared to the response facilitated by CMC. The 200 µg/mouse dose of CNC also caused significant increases in serum ALT and AST at 7-days post-exposure compared to controls. CMC exposure also facilitated a significant increase in serum AST at the 200 µg/mouse dose at 1-day post-exposure. MWCNT resulted in significant pulmonary inflammation. However, at 1-day post-exposure, CNC (50 µg/mouse) caused a 2-fold greater increase in PMN number compared to that by MWCNT. By 7-days post-exposure MWCNT caused a 3-fold greater increase in PMN number compared to CNC. Additionally, there was no treatment related changes in sperm count or motility for any dose/post-exposure time combination of CNC, CMC, or MWCNT. In conclusion, the physico-chemical properties, such as the enhanced surface area of the CNC, are likely driving the more robust pulmonary toxicity response as compared to the pulmonary toxicity caused by the larger micron-sized CMC. Furthermore, MWCNT-facilitated pulmonary inflammation that was more persistent than CNC.

2111 Pulmonary Responses to Subacute Cadmium Sulfide Nanoparticle Inhalation

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The uses and applications of engineered nanomaterials (ENMs) are expanding at an astounding rate. One such application is the manufacturing of quantum dots using cadmium sulfide (CdS) nanoparticles. CdS quantum dots are semiconductors with a direct intermediate bandgap and excellent thermal stability and thus have shown strong potential for wide scale use in solar cells, light emitting diodes, and specialty lasers. Industrial scale manufacturing of CdS quantum dots requires bulk quantities of CdS nanoparticles. To evaluate toxic risk associated with industrial CdS nanoparticle use, we performed a sub-acute nose-only inhalation exposure (3.49 ± 0.49 mg/m^3) to CdS aerosol (geometric mean mobility diameter 41 nm, geometric standard deviation 1.8) using a murine model. Analysis of bronchoalveolar lavage (BAL) fluid showed a 12-fold increase in total BAL cells per mouse immediately after 2 wk exposure accompanied with inflammatory cell infiltration (41% of these cells were neutrophils) that persisted through 3 weeks of rest after exposure (the number of neutrophils decreased only to 29%). Histopathologic analysis of lung tissue confirmed the presence of inflammatory cell infiltration. Exposed mice demonstrated an increase in inflammatory cytokines in the BAL fluid. There was evidence of lipid peroxidation in both lung tissue and serum of exposed mice (TBARS assay). Our analysis shows that sub-acute inhalation of CdS nanoparticles leads to pulmonary inflammation and an increase in ROS. Determination of Cd in selected tissues to assess deposited and cleared Cd after exposure as well as evaluation of pulmonary mechanics parameters is in progress. *Supported by: NIH U01 ES027252 & NIH P30 ES005605.*

2112 Effects of Iron Oxide Nanoparticles (IONPs): Cytotoxicity and Genotoxicity in Wistar Rat Brain

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The utilization of iron oxide nanoparticles in several biomedical applications, has received much attention due to their unique properties, such as extremely small size, high surface-area-to-volume ratio, excellent magnetic properties and great biocompatibility. Although, the potential benefits of iron oxide nanoparticles are considerable, there is a distinct need to identify any potential risk associated with their usages. It has been demonstrated earlier that corpus striatum and hippocampus are the major sites of IONPs accumulation in brain. The high surface activity of these IONPs deposited for long term in brain may cause neurodegenerative disorders. Our study put forward a notion that in making of a perfect treatment vehicle, any nanoparticle has to pass through various parameters of cytotoxic and genotoxic evaluation. Thus, the present study was designed to evaluate the cytotoxic and genotoxic effects of iron oxide nanoparticles (IONPs) *in vivo*. In order to study the toxic effects, male Wistar rats (6-8 weeks old) were randomly divided into four groups, group I served as control, group II treated with 20.322 mg/kg body weight, group III treated with 40.644 mg/kg body weight and group IV treated with 81.288 mg/kg body weight. Animals of each group were intraperitoneally administered the selected dose daily for 28 days. They were sacrificed on post exposure day 7th, 14th and 28th and brains dissected out for various biochemical assays such as antioxidant enzymes activity glutathione-S-transferase (GST), glutathione peroxidase (GPX), and glutathione reductase (GR), and genotoxicity assessment through single cell gel electrophoresis assay, in the tissue homogenates of four brain sub regions namely, frontal cortex, corpus striatum, hippocampus and cerebellum. The results revealed that the activity of antioxidant enzymes (GST, GR, GPx) elevated in IONPs treated groups in dose-dependent manner but non-significantly, however, activity of GR and GPx on 14th day of investigation for group IV animals was found significant. The comet assay results indicate that IONPs did not induce any significant DNA damage following exposure of IONPs at various dose levels. The present study concluded that IONPs are safe to use under prescribed doses. But it can induce oxidative stress as well as genotoxicity at higher doses of the particles with or without coatings. Therefore, there is a considerable need to address biocompatibility and biosafety concerns associated with their usage to avoid the adverse side effects of nanoparticles when used as biomedical tools.

2113 Time Course of Pulmonary Toxicity and Biodistribution during and after Subacute Inhalation Exposure to Copper Oxide Nanoparticles in a Mouse Model

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Research interest in nanotoxicity has dramatically increased due to the exponential growth of nanomaterial applications and nanotechnology because of their unique physicochemical properties compared to bulk materials. Copper oxide nanoparticles (CuO NPs) have been widely used in many applications such as antimicrobial agents, solar cells, catalysts, and electronic products. CuO NPs are highly cytotoxic compared to other metal oxide NPs. The inhalation is potentially the most significant route for unintentionally exposure especially in occupational settings. Several murine studies demonstrated that inhalation exposure to CuO NPs induces pulmonary inflammation. However, available data on the CuO NPs biodistribution relating to their toxicity are still limited. The goal of this study was to investigate the pulmonary toxicity and biodistribution of inhaled CuO NPs during and after subacute exposure. The time course of CuO NPs biodistribution will increase our understanding of CuO NP toxicity particularly in the pulmonary region. In this study, female BALB/CJ mice were exposed to CuO NPs at 3.75 mg/m^3 (geometric mean of aerosolized CuO NPs was 77.6 nm) using a nose-only exposure system for 4 hr/day, 5 days/week over a 2-week period and were necropsied on days 0, 3, 7, 10, 12, 17, 22, and 27. Sera, bronchoalveolar lavage (BAL) fluid and lung tissue were collected to measure 23 cytokine/chemokines levels, numbers of inflammatory cells, and lactate dehydrogenase (LDH). Histopathology of lungs was also evaluated. Whole blood, lung, brain, heart, kidney, liver, and spleen were collected to measure copper concentration by inductively coupled plasma mass spectrometry (ICP-MS). The number of macrophages, lymphocytes and neutrophils in BAL fluid were gradually increasing with a culmination on day 22, 17 and 12, respectively. Rate of weight gain, and LDH levels in BAL fluid exhibited similar changes as neutrophils. Significantly higher concentrations of many cytokines/chemokines (IL-12 (p40), KC, MCP-1, MIP-1a) persisted several days after the end exposure. The amount of copper in the lung tissues of all exposure groups increased significantly compared to the control group with a gradual increase observed during exposure and a decline subsequent to exposure. However, the copper concentration in whole blood exhibited a

significant increase only on day 7 and 17 compared to the control group. The copper concentration in other remaining organs will be further measured to assess the copper translocation. *Funded by: NIH U01 ES027252.*

2114 Toxicity of Multiwalled Carbon Nanotubes in Rat and Human Intestinal Cell Models

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Multi-walled carbon nanotubes (MWCNTs) are several layers of one-atom thick sheets of carbon. MWCNTs are hollow cylinders with a large length to diameter ratio. MWCNTs can improve the mechanical, electrical and thermal conductivity of many products so they are used in a wide variety of applications. The manufacture and use of MWCNTs may result in their release in to the environment. Exposure to MWCNTs may occur following inhalation and ingestion of these nanomaterials. The purpose of this study was to assess the *in vitro* toxicity of several MWCNTs in a rat (IEC-6 cells) and human intestinal cell models. The outside diameter (OD) of the MWCNTs were < 8 nm, 13-18 nm, 20-30 nm, and >50 nm. Two other MWCNTs of OD 20-30 nm were functionalized with -OH or -COOH groups. The IEC-6 cells are a 2-dimensional monolayer of cells, whereas the human model is a 3-dimensional model with multiple cell types. IEC-6 cells were plated in 96-well plate with 60K cells/well for 24 h. MWCNTs suspended in Dulbecco's media, 10% fetal bovine serum (FBS) and 0.1% pluronic were probe sonicated for 15 min before dosing. Media with 10% FBS was the negative control and Triton X-100 (0.3%) was the positive control. Cells were then exposed to the MWCNTs at several concentrations (0.3-300 µg/mL) for 24 h. Following incubation, the cells were washed with media and the cytotoxicity was assessed using a cell-permeant dye, Calcein AM. This is a non-fluorescent compound that is converted to the green fluorescent Calcein in viable cells upon acetoxymethyl ester hydrolysis by intracellular esterases. Lethal concentration$_{50}$ of the MWCNTs were determined: < 8 and 20-30 nm -OH, 35 µg/mL; 20-30 nm, 50 µg/mL; 20-30 nm -COOH, 80 µg/mL; 13-18 nm, 105 µg/mL; 50 nm, 300 µg/mL. Cell viability measured in the 3-dimensional human intestinal model using the colorimetric MTT assay showed no cytotoxicity by the MWCNTs following a 24-h exposure. For the rat intestinal model, outside diameter and functionalization of MWCNTs appear to be important factors in the cytotoxicity of these nanomaterials. *This abstract does not represent US EPA policy.*

2115 Titanium Dioxide and Zinc Oxide Nanomaterials Change Lipid Order and Increase Permeability in Model Systems

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The expanded use of nanotechnology has led to increased production and use of engineered nanomaterials (ENM), resulting in an increased risk of human exposure. ENM generated occupationally can be airborne and are taken-up by immune cells (macrophages) in the lung, where the ENM accumulate in phagolysosome organelles. Some ENM have been reported to be bioactive and to trigger a pro-inflammatory response when inhaled. Alveolar macrophages have been demonstrated to be responsible for this inflammatory response due to their release of the cytokine, IL-1β. A key step preceding and linked to IL-1β release is phagolysosomal membrane permeability (LMP). This suggests that ENM may interact directly with the lipid membrane of the phagolysosomes, disrupting their normal state, resulting in LMP. The way in which various ENM disrupt lipid membranes, is not fully understood. Time-resolved fluorescence anisotropy measurements, using suitable lipid probes, can measure changes in membrane characteristics, such as lipid order (Lo) and disorder (Ld). Using appropriate model systems can help to describe mechanisms of ENM0-induced lipid membrane permeability. In this work, 100 and 400 nm liposomes made of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine) and DOPS (1,2-dioleoyl-sn-glycero-3-phospho-L-serine) were used as model systems to determine phospholipid interaction with ENM. These models were exposed to 25 and 100 µg/ml of titanium dioxide (TiO_2) or zinc oxide (ZnO) nanospheres for 2 hours at 37°C. The fluorescence membrane probe Di-4-ANEPPDHQ and a time-resolved fluorometer were used to determine the changes in lipid Lo/Ld of the liposomes by anisotropy. Liposome lysis was measured by a calcein leakage assay. Again, these liposomes were exposed to the same doses and incubation time as described above. Treatment with both ENM produced significant increases in calcein leakage at the 100 µg/ml dose. This happened in all three models tested. POPC liposomes exposed to 100 µg/ml TiO_2 had a decrease in lipid order, but no significant change was observed using DOPC or DOPS liposomes. ZnO exposure (100 µg/ml) to DOPS liposomes also showed a decrease in lipid order, but again there was no change using DOPC or POPC liposomes. These results indicate that there is a consistency in term of changes to the order of the membranes, in which there is increased order around the probe. These results also demonstrate that both of these ENM are able to cause leakage of the small molecule calcein from inside the liposomes. *Funding: NSF MRI CHE-1531520 M.J. Murdock Charitable Trust, NIH R01ES023209, 1F32ES027324, P20GM103546 and P30GM103338.*

2116 Oxygen Mediates the Toxicity of Silver Nanoparticles in Human Liver Cells

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Due to the increasing concern of their hazard effects, there has been a rapid progress in characterizing the toxicological profiles of nanosilver (nAg). The current knowledge of the toxic effects, however, was mainly gained in the normoxic experimental condition, and only limited information is available on the effects of nAg in hypoxic conditions. Hypoxia is commonly found in humans in various clinical conditions, such as stroke, chronic injury, chronic wounds and solid tumors. There are several known scenarios where nAg exposure and hypoxia occur concurrently, such as nAg treated wound sites, nAg incorporated cancer therapy, and nAg administration to chronically diseased tissue. Therefore, investigating the effects of nAg in combination with hypoxia, addresses the toxicity concern in close mimic of these conditions. Such investigation also adds additional information to the overall understanding of nAg potential adverse effects in general. In this work, cytotoxicity, proliferative activity, oxidative stress and apoptotic potential of silver nanoparticles (AgNPs), were evaluated under both hypoxic (2% O_2) and normoxic (21% O_2) conditions. Two human liver cell types, including cancer cells (HepG2) and non-cancer cells (THLE-3) were tested. Results revealed that the toxicity of AgNPs was dependent on both O_2 level and cell type. AgNPs were more toxic to cancer cells in normoxia, whereas it's the opposite to non-cancer cells. AgNPs were only toxic to HepG2 above 5 mg/L during 24-hour exposure regardless of O_2 availability. At 20 mg/L AgNPs in normoxia, 1) cells were 50% less viable, 2) AgNPs exerted up to a 3-fold response in oxidative stress and 3) 20% more apoptotic cells compared to hypoxia condition. THLE-3 cells, on the contrary, were severely damaged in addition to insufficient O_2. At 1 mg/L AgNPs and 2% O_2, 1) cells were 30% less viable, 2) 20% fewer in population, and 3) showed non-significant increased apoptosis. Meanwhile, cellular uptake is being evaluated to study the link between toxicity and uptake amount. The protein expression of hypoxia-inducible factor (HIF-1α), an oxygen-sensitive transcription factor, will also be measured in the presence and absence of AgNPs under both O_2 conditions, to elucidate the underlying mechanism of hypoxia on AgNPs-induced toxicity. Overall, toxicity of AgNPs to human liver cells is mediated by O_2 level at which the exposure occurs. Hypoxia ameliorates AgNPs toxicity to cancer cells, while exacerbating it to non-cancer cells.

2117 The Effects of Genotype × Phenotype Interactions on Transcriptional Response to Silver Nanoparticle Toxicity in Organotypic Cultures of Murine Tracheal Epithelial Cells

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The airway epithelium is critical for maintaining innate and adaptive immune responses, and occupational exposures that disrupt its immune homeostasis may initiate and amplify airway inflammation. We have demonstrated that silver nanoparticles (AgNP), which are used in multiple applications including many antimicrobial products, induce toxicity in organotypic cultures derived from murine tracheal epithelial cells (MTEC). Those cells differentiated toward a "Type 2 [T2]-Skewed" phenotype experienced an increased sensitivity to AgNP toxicity, suggesting that asthmatics could be a sensitive population to AgNP exposures in occupational settings. However, the mechanistic basis for this genotype × phenotype (G×P) interaction has yet to be defined. In this study, we conducted transcriptional profiling using RNA-sequencing to predict the enrichment of specific canonical pathways and upstream transcriptional regulators to assist in defining a mechanistic basis for G×P effects on AgNP toxicity. Organotypic cultures were derived from MTEC across 2 genetically inbred mouse strains (A/J and C57BL/6J mice), 2 phenotypes ("Normal" and "T2-Skewed"), and 1 AgNP exposure (an acute 24 h exposure) to assess G×P effects on transcriptional response to AgNP toxicity. The "T2-Skewed" phenotype was marked by increased pro-inflammatory T17 responses to AgNP toxicity, which are significant predictors of neutrophilic/difficult-to-control asthma and is compatible with our hypothesis. This study

highlights the importance of considering G×P effects when identifying sensitive populations, whose underlying genetics or diseases could directly modify their response to AgNP exposures.

2118 The Yin-Yang of Inflammation: Is There a Balance during Nanoparticle Toxicity?

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Nanoparticle exposure is rising due to their versatile utility as drug carriers, mechanical property enhancers in medical devices, consumer products, and food excipients. To name a few multiwalled carbon nanotubes (MWCNT), cobalt oxide (cancer therapy), silver (antibacterial composition), titanium oxide (sunscreen), are routinely used in a variety of formulations. Nanoparticles can accumulate in tissues due to their extremely small size and elicit toxic effects. Despite the tremendous benefit presented, common pitfalls of this technology are its potential short and long-term effects on the human body. High mobility group box-1 (HMGB1) acts as a damage-associated molecular pattern and can work as a necrosis signal for the immune cell activation through the toll-like receptor (TLR). HMGB1 can bind to multiple receptors including TLRs in liver diseases. Our previous differential HMGB1 expression analysis following TiO_2 or MWCNT exposure showed an increase in HMGB1 protein expression in the hepatocyte cell line, HC-04. Tumor necrosis factor α induced protein 3-interacting protein 1 (TNIP1), a cytoplasmic protein, inhibits signaling mediated by numerous trans-membrane receptors, such as TLRs, thus acting as a negative regulator of TLR signaling. Both HMGB1 and TNIP1 converge at TLR signaling: where HMGB1 activates TLRs producing an inflammatory response and TNIP1 restricts TLR mediated inflammation. Thus, in this analysis, our goal was to evaluate the relationship between HMGB1 expression and TNIP1 expression in HC-04 cells exposed to nanoparticles. Interestingly, we evidenced an inverse relation between HMGB1 and TNIP1 protein expression. Hepatotoxicants such as acetaminophen (APAP) and ethanol (EtOH) have been shown to induce HMGB1 expression in the liver. In HC-04 cells, HMGB1 expression also increased due to APAP and EtOH whereas TNIP1 expression decreased. For the first time, we could demonstrate the changes in TNIP1 expression after a xenobiotic exposure and its relationship with a pro-inflammatory signal. In summary, the results suggest that nanoparticle exposure results in an increase in the inflammatory ligand, which in combination with decreased inhibitory control on inflammatory signaling may predispose cells to the inflammatory response to otherwise less toxic xenobiotic exposure.

2119 Formation of Low Molecular Weight Products during Environmental Transformation of Graphene Oxide Modulates Toxicity in Aquatic Species

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Carbon-based nanoparticles (CNPs) are used extensively in industrial, consumer, and mechanical applications. Their unique structural properties provide novel opportunities to develop more robust and innovative commercial products including paints, fabrics, cosmetics and electronics. With increasing commercial use of CNPs, environmental exposure is growing increasingly common. Specifically, graphene oxide (GO) has been shown to compromise cell integrity via interactions with lipid membranes and subsequent induction of oxidative stress *in vitro*. An understanding of GO-membrane interactions and resulting potential of GO to perturb biological systems are crucial for estimating risk. To assess the exposure and toxicological implications of environmental transformations of GO in aquatic species, GO suspensions were photo-irradiated by simulated sunlight for up to 490 hrs. Fathead minnow (FHM) epithelial cells were exposed to both GO/reduced (rGO) suspensions as well as their filtrates. The formation of low molecular weight products (LMWPs) was assessed by mass spectrometry and metabolomic profiling was used to investigate the biological response in this ecologically relevant cell line. GO readily undergoes both direct and indirect photo-transformation processes and increasing time of irradiation increases the biological response of FHM cells. Both decreased size of GO (or rGO formation) as well as the formation of LMWPs are likely contributing to this cellular response. In FHM cells, GO induced changes in numerous markers of oxidative stress. Most biological pathways affected included perturbations in the citric acid cycle, glycolysis and amino acid metabolism. Removal of suspended GO/rGO via filtration still elicited a biological response in our test system. Mass spectral identification of the LMWPs suggested that the photo-production of polycyclic aromatic hydrocarbon (PAH)-like derivatives facilitate the measured response in FHM cells. Ultimately, identifying potential biomarkers of GO exposure and the development of exposure indices of GO and its photoproducts in human and ecologically relevant species will aid in accurately establishing risk.

2120 Liposomal Composition Can Affect Nanoparticle Transport into Epithelial and Endothelial Cells

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Biologically compatible liposomes have value as vehicles for drug delivery. Potential capability of liposomes to cross membrane barriers of epithelial, endothelial, and astrocytic cells was examined using liposomes alone, liposomes with cholesterol, and liposomes with cholesterol and folate. The liposomes were composed of phosphatidylcholine derivatives and surfactants. FITC dye-labeled products were examined in transwell cultures of Caco-2 epithelial cells, HBEC-5i endothelial cells, and astrocyte cultures. With DMSO as a positive control, none of the liposomal products at 10 micromolar concentrations contributed to cell death in any of the cell cultures when examined 6 h after exposure. Cellular uptake measured at 4 h and 24 h demonstrated 34% and 88% of HBEC-5i had taken up the liposomes alone. Uptake of liposomes was 7.8% at 4 h and 34% at 24 h by astrocytes and 18% at 24 h by Caco-2 cells. Liposomes containing cholesterol with or without folate did not take up these FITC-labeled products in any of these cells. Liposomes with folate but without cholesterol resulted in 34% and 30% uptake, respectively, in Caco-2 and HBEC-5i cells after 24 hours. These results suggest that, for this particular liposomal nanomaterial, inclusion of cholesterol was detrimental to cellular uptake. *Supported at Virginia Tech by Luna Innovations, Inc.*

2121 Dermal Toxicity of Nickel- and Cobalt-Based Nanocatalysts

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Nanocatalysis is a fast-growing field involving the use of nanomaterials as catalysts for a variety of applications, specifically, metal nanoparticles (NP) and their compounds, which have a large surface-to-volume ratio compared to bulk materials. Metal/metal oxide (Me/MeO) NP possess unique properties which can be useful in different applications including catalytic processes such as decomposition, reactions of dehydrogenation, oxidation, alkylation, C-C coupling, among others. Nonetheless, the same properties that make these metal nanocatalysts (NCT) very attractive can pose potential health risks. In addition to inhalation exposure route, workers may also be exposed through skin contact. In this study, we evaluated the ability of four different NCT ($NiFe_2O_4$, $CoFe_2O_4$, Ni and Co_3O_4) to initiate oxidative stress, induce redox-sensitive transcription factors and to trigger inflammation in primary human epidermal keratinocytes (HEK). Besides, due to the skin's vulnerability to UV radiation, it is important to assess whether metal NCT augment the adverse effects of UVB. HEKs exposure to the studied Me/MeO NCT (0-20 μg/cm^2) resulted in a dose- and time-dependent reduction in cell viability, cell damage, activation of NF-κB, elevated ROS generation, release of inflammatory mediators, and increase in oxidative stress markers. Co-exposure of HEK to UVB (4KJ/m^2) and Me/MeO caused marked amplification of the observed responses. UVB exposure alone induced significant cytotoxicity and secretion of cytokines/chemokines. Based on the hierarchical clustering analysis of the cytokine/chemokine responses, Co_3O_4 and Ni were segregated from the control and both ferrites-exposed samples. Pre-treatment with UVB resulted in separation of Ni, Co_3O_4 and $NiFe_2O_4$ responses from control and $CoFe_2O_4$ exposure groups. Overall the inflammatory responses in HEK cells induced by exposure to different Me/MeO NPs investigated, with or without UVB pre-treatment, were in order: Ni>Co_3O_4>$NiFe_2O_4$>$CoFe_2O_4$. Altogether, these data indicated that co-exposure of dermal cells *in vitro* to Me/MeO NP and UVB was associated with potentiation of the adverse effects as compared to the cells treated with NCT alone. *Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.*

2122 Silver Nanoparticle-Mediated Mast Cell Degranulation Metabolic Pathway Shifts Are Distinct from IgE-Mediated Degranulation

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Silver nanoparticles (AgNPs) are incorporated into a variety of consumer and medical products, primarily due to their antimicrobial properties, thereby leading to increased exposure in the general population. We have previously demonstrated that 20 nm AgNPs induce robust mast cell degranulation through a novel non-IgE mechanism. Mast cells are important effector cells in the immune system and are essential to an allergic response. To better understand this novel mechanism of mast cell degranulation, we characterized and compared cellular metabolic shifts across several mechanisms of degranulation (AgNP, IgE, compound 48/80 [non-IgE]) in murine bone marrow-derived mast cells (BMMCs). To explore these metabolic changes, we utilized functional assays to measure glycolysis and aerobic mitochondrial respiration in response to treatments. We observed an increase in ATP production which correlated to degranulation, however, differing levels of mitochondrial dysfunction and glucose uptake were observed between AgNP and other treatments. In addition, we utilized Seahorse XFp technology to measure: 1) preferential metabolic pathway phenotypes, 2) glycolytic metabolism, and 3) mitochondrial respiration metabolism. The cell phenotype test revealed that BMMCs shift away from glycolysis when degranulated via AgNP whereas IgE mediated degranulation preferentially utilized glycolysis. The Cell Mito stress test revealed a decrease in respiration for all mechanisms of degranulation, compared to control, with consistently lower levels for non-IgE degranulation. Compound 48/80, but not AgNP, also caused complete inhibition of glycolytic mitochondrial respiration reserve. We next observed a decrease in glycolysis that was most prominent in non-IgE degranulation. There was a complete depletion of glycolytic reserve, similar to mitochondrial respiration, with non-IgE degranulation (AgNPs and compound 48/80) that did not occur with IgE mediated degranulation. In conclusion, mast cell metabolism varies significantly between AgNP degranulation and IgE mediated degranulation suggesting novel cell regulatory mechanisms are potentially driving AgNP mediated mast cell degranulation.

2123 Potential Mitochondrial-Targeted Toxicity of Silver Nanoparticles in Mouse Hepatocytes

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Silver nanoparticles (AgNPs) are a well-proven antimicrobial nanomaterial, which hold great promise for a wide range of biological applications. Despite the promising advantages, the significant human exposure to AgNPs has given rise to concerns over potential human health and environmental hazards. While multiple studies have investigated the toxic effects of various AgNPs at different levels, the underlying mechanism is still largely elusive. In this work, we explore the possible relationship between mitochondrial activity and the cytotoxicity of AgNPs with different coating and sizes, compared to silver ions, in cells cultured in glucose and galactose-based media. Since cells cultured in galactose rely mostly on oxidative phosphorylation (OXPHOS) to produce their ATP, they become more sensitive to mitochondrial toxicants than cells grown in glucose medium. Analysis of bioenergetic function with the XF Seahorse extracellular flux analyzer further confirmed that oxygen consumption rate (OCR) was significantly increased whereas extracellular acidification rate (ECAR), a measure of glycolysis, was decreased in cells grown in galactose. Cytotoxicity assays, mitochondrial stress analyses, and fluorescence and darkfield microscopy were used to investigate the effects of AgNPs on cell proliferation and metabolism. Our data suggest the sensitivity of mitochondria to AgNPs, which could in turn have an impact on cell viability and proliferation.

2124 *In Vitro* Pulmonary Toxicity of Copper Carbonate ($CuCO_3$) Particles Used as Outdoor Wood Preservatives

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Micronized $CuCO_3$ containing nanoparticles (NPs) are employed as outdoor wood preservatives. This application raises a risk of human exposure to $CuCO_3$ NPs or their products with unknown health effects. Research examined the *in vitro* pulmonary toxicity of milled $CuCO_3$ particles and saw dust extracts (SDE) obtained from $CuCO_3$ treated and untreated wood. A thiobarbituric acid reactive substance (TBARS) assay measured the oxidative reactivity of all samples with and without the copper chelator triethylenetetraamine dihydrochloride (TETA). Analysis of milled $CuCO_3$ samples demonstrated samples containing the smallest size distribution displayed the greatest TBARS reactivity. SDE from only $CuCO_3$ treated wood displayed TBARS reactivity similar to milled $CuCO_3$ samples. TBARS reactivity of milled $CuCO_3$ and SDE from treated wood was eliminated after filtration through a 3kDa filter. TETA significantly attenuated TBARS reactivity of milled $CuCO_3$ samples but not SDE from treated wood indicating reactivity was not due to ionic Cu in the $CuCO_3$ treated wood. BEAS2B cells were exposed to either milled $CuCO_3$ samples (25 - 200 µg/ml) or SDEs (2.75 - 88 mg/ml) and cytotoxicity assessed using the WST1 assay at 22h post-exposure. Milled $CuCO_3$ samples with the smallest size distribution were most cytotoxic to BEAS2B cells. SDE from treated wood was more cytotoxic when compared to SDE from untreated wood. TETA attenuated BEAS2B cytotoxicity for both milled $CuCO_3$ particles and SDE from treated wood. On a molar dose metric, SDE from treated wood was more cytotoxic to BEAS2B cells compared to milled $CuCO_3$ samples. BEAS2B IL-6, IL-8, and HO-1 gene expression was examined by qRT-PCR after 3, 6, 12, 24, and 48h exposure to milled $CuCO_3$ samples or SDEs. Milled $CuCO_3$ and SDE samples induced proinflammatory and stress gene expression to varying extents in a time dependent manner and did not correlate with TBARS reactivity. Milled $CuCO_3$ particles elicited a completely different gene expression profile when compared to SDE from treated wood. These findings highlight the challenges in assessing the *in vitro* pulmonary toxicity of $CuCO_3$ treated outdoor wood samples using milled $CuCO_3$ with similar size distribution. The results indicate *in vitro* pulmonary toxicity should be assessed using the nano-enabled product. *This abstract does not represent US EPA policy.*

2125 Biological Effects of Long-Term Exposure of Human BEAS-2B and Met-5A Cells to Riebeckite/Tremolite Asbestos and Their Respective Cleavage Fragments

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Asbestos is a commercial term that refers to 6 different fibrous minerals including riebeckite (RF) and tremolite (TF) amphibole asbestos. Inhalation of respirable forms of amphibole fibers, can cause asbestosis, lung cancer and both pleural and peritoneal mesothelioma. Amphiboles can also occur in a non-fibrous habit that can be mechanically broken into cleavage fragments (CF) which can meet the mineralogical/regulatory criteria for fibers. While the effects of RF and TF on health are well documented, there is uncertainty regarding the toxicity of riebeckite and tremolite CF. In this study, human epithelial (BEAS-2B) and mesothelial (MET-5A) cells were evaluated for the presence of several cancer hallmarks indicating the neoplastic-like transformation following continuous long-term (5 weeks) exposure to sub-toxic (2.5µg/cm^2) concentrations of RF, TF and their CF. TF- and RF-exposed cells, both BEAS-2B and MET-5A, revealed a neoplastic-like transformation phenotype characterized by significant increase in invasion/migration, anchorage-independent growth, proliferation, and morphological transformation, compared to controls. No anchorage-independent growth and invasion was observed in both cell types treated with riebeckite CF although an increase in DNA damage, migration, proliferation, and morphological changes were detected in BEAS-2B cells. In the case of tremolite CF, although a significant increase in proliferation, transformation and DNA damage was observed in both cell types, an increase in invasion/migration and anchorage-independent growth was detected only in BEAS-2B cells. Similarly, analysis of inflammatory responses suggested cell-type specific effects as well as treatment related differences. Overall, our data are compatible with the findings that amphibole asbestos fibers demonstrate higher neoplastic transformation potential compared to the respective CF (at the same mass dose) in both bronchial epithelial and mesothelial cells.

2126 Advanced *In Vitro* Models for the Assessment of Nanomaterial-Induced Lung Fibrosis

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Nanotechnology promises significant scientific, economic and societal benefits, but commercialization and growth are threatened by safety uncertainties. Existing *in vitro* hazard testing strategies to define the human health impact of nanomaterials (NM) commonly apply unrealistic acute, high-doses to cellular models that poorly reflect the *in vivo* environment. The H2020 project "*Physiologically Anchored Tools for Realistic nanOmateriaL hazard aSsessment*" (PATROLS) aims to establish innovative hazard assessment tools to predict adverse effects caused by long-term NM exposure, and support regulatory risk

decision making. In this project, we focus on the evaluation of the pro-fibrotic activities of NM and develop advanced *in vitro* models for the assessment of NM-induced lung fibrosis. Based on the central role of fibroblasts in lung fibrosis, we developed a strategy of fibroblast exposure mimicking the pulmonary environment of this cell type during inhalation exposure by assessing the responses to a direct contact of fibroblasts with NM, or indirect effects of NM on fibroblasts via their crosstalk with epithelial and inflammatory (mainly macrophages) cells. Pro-fibrotic responses to NM are examined in human lung fibroblast cell lines (MRC-5 and CRL1490) acutely (24 h) and chronically (8 weeks) exposed to low (realistic) doses of NM. Fibroblasts will also be treated with supernatants of epithelial and inflammatory cells (cultured alone or in co-culture) acutely or chronically exposed to NM to mimic the indirect activity of NM. Acute and chronic, as well as direct and indirect effects, will be compared to identify the modes of action of the tested NM. As we previously identified the activation of fibroblasts as a relevant key event with a high predictive value for lung fibrosis [1], we will focus on fibroblast proliferation, differentiation, collagen production, etc. The predictive value of the different models will be evaluated by comparing NM able to induce lung fibrosis *in vivo* (e.g. multi-wall carbon nanotubes, MWCNT) to non-fibrotic NM (e.g $BaSO_4$). High aspect ratio NM (MWCNT) will also be compared to low aspect ratio NM able to induce lung fibrosis (e.g. CeO_2). *In vitro* models and conditions are currently under development and preliminary results will be presented. 1. Vietti, G., D. Lison, and S. van den Brule, *Mechanisms of lung fibrosis induced by carbon nanotubes: towards an Adverse Outcome Pathway (AOP).* Part Fibre. Toxicol, 2016. 13(1): p. 11.

2127 Cytotoxicity of Engineered Nanomaterials on Primary Epithelial Cells in Air-Liquid Interface Culture

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The unique physicochemical characteristics of engineered nanomaterials (ENMs) have led to their increased applications in pharmaceuticals, biomedical devices, and consumer products. ENMs range from 1-100 nm in at least one dimension and possess unique properties at an atomic level. They are often used to increase the quality of consumer products due to their stability, thermal conductivity, and mechanical properties and exhibit different properties depending on their size and surface area. Graphene is known for its optical transmittance and chemical inertness; graphene oxide and reduced graphene oxide nanoparticles have been incorporated in biomedicine for biosensing and drug delivery. Copper oxide nanoparticles exhibit high thermal conductivity, stability and potentially antimicrobial properties. The versatility and increasing use of ENMs raises concerns for human health since inhalation can be a major route of exposure with potential mechanisms of toxicity not well understood. Because ENMs vary in size, structure, and chemical properties, it is difficult to assess which characteristic is associated with biological toxicity. In order to shed light on the role of different physicochemical properties in respiratory toxicology *in vitro*, we exposed primary mouse tracheal epithelial cells on an air liquid interface (ALI) culture to well characterized ENMs of different chemical properties and sizes. These ENMs include: copper oxide, cadmium sulfide, molybdenum disulfide, hexagonal boron, graphene oxide in water, graphene oxide (250 nm), graphene (110 nm), and reduced graphene oxide. Cells were grown in culture until confluent and fully differentiated, then exposed to ENMs at concentrations of 125 or 250 µg/mL for 24 hours. Because assays for measuring cell number metabolically are prone to particle interference, cytotoxicity was determined by fluorescent microscopy on transwell membranes. Our results showed that copper oxide, hexagonal boron, graphene (110 nm), and reduced graphene oxide at a concentration of 250 µg/mL were cytotoxic as well as graphene oxide in water at 125 µg/mL. The remaining ENMs tested did not cause cytotoxicity. We conclude that this method can be used to study ENM toxicity and that copper oxide nanoparticles are cytotoxic to large airway epithelial cells. *Supported by U01 ES027288.*

2128 Exposure to Nanoparticles in a Food Matrix Alters Intestinal Function in an *In Vitro* Model

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Overall well-being is related to gut health and function. The gastrointestinal (GI) tract forms a selective barrier that protects the host from harmful luminal content and allows essential nutrients and water to pass into circulation. Food additives and microbial dysbiosis may influence intestinal health and activity of intestinal alkaline phosphatase (IAP), a gut mucosal defence factor, while playing a role in the development of GI disorders. An average of 10^{12}-10^{14} edible metal oxide nanoparticles (NP) are consumed per day. In this study, a Caco-2 and HT29-MTX E12 intestinal model of the GI tract was used to study the effects of food-grade titanium dioxide NP in a food matrix (FM) on intestinal permeability, IAP activity, and nutrient transport. The NP-microbiota-gut interaction was tested by including Gram-positive, commensal (*LactoBacillus rhamnosus*, *Bifidobacterium bifidum*) or Gram-negative, opportunistic (*Escherichia coli*) bacteria into the model. The model was subjected to 10^3 CFU/mL of bacteria and physiological doses of digested TiO_2 NP in FM. The FM was a semi-synthetic meal prepared with all the essential components of a regular diet. Cell membrane permeability was assessed by a Lucifer Yellow (LY) permeability assay. Glucose, protein and fatty acid transport were assessed using colorimetric assays. Post-exposure, bacterial cell viability was assessed with a drop plate method. While FM did not affect the barrier integrity, *E. coli*+TiO_2 NP in FM showed a decrease in permeability ($p<0.0001$) and IAP activity ($p<0.001$) compared to no bacteria condition. TiO_2 in FM led to a decrease in protein and triglyceride transport across the barrier. *E. coli* remediated this effect of NP. Glucose transport was unaffected by the NP. A permeable intestinal barrier can lead to systemic inflammation and potential tissue damage. The results of this study suggest that while TiO_2 affects protein transport, *E. coli* can ameliorate the changes. Dietary conditions influence the attachment of bacterial cells to the cell monolayer, further affecting the intestinal function. These results highlight the complex interaction of the microbiome, diet, and gut function. With the use of multiple human intestinal cell types, human-derived bacteria and digestion, our *in vitro* model provides a physiologically relevant method to investigate these interactions.

2129 Mechanisms of Rare Earth Metal Oxide Nanoparticle Toxicity in Macrophages

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Rare earth metal oxide nanoparticles (REMO NPs) are synthesized from lanthanide series metals complexed with oxygen. REMO NPs are desired due to their unique chemical, catalytic, electrical, magnetic, and luminescent properties for applications in both material science and medicine. Occupational exposure to REMO NPs may occur through inhalation during their manufacturing. Additionally, biomedical use of these nanoparticles is increasing therefore it is important to understand their toxicity. Further, due to their novelty, the toxicity of these particles is not well characterized. Recently, several studies have shown adverse effects associated with exposure to REMO NPs including pulmonary inflammation *in vivo* and cytotoxicity *in vitro*. We hypothesized that toxicity of REMO NPs is due to their unique physicochemical properties and would elicit adverse immune responses in macrophages. Ten different REMO NPs were screened for cytotoxic effects by dosing the RAW 264.7 macrophage cell line with 1-50 µg/mL for 24 hours. Four nanoparticle types were selected for further testing and characterization based on differences in cytotoxicity profiles. These four were lanthanum oxide (La_2O_3) (high toxicity), gadolinium oxide (Gd_2O_3) (medium toxicity), neodymium oxide (Nd_2O_3) (medium toxicity), and europium oxide (Eu_2O_3) (low toxicity). Using these four REMO NPs, we further investigated the correlation of physicochemical properties to cytotoxicity and activation of macrophages including reactive oxygen species generation and cytokine production. It was found that hydrodynamic size and the amount of REMO NP uptake by macrophages directly correlated to cytotoxicity, mitochondrial disfunction, and inflammatory responses, thus, linking the physicochemical properties of REMO NPs to their effect on macrophages. In conclusion, the safety profile of REMO NPs should be further evaluated prior to incorporation in biomedical applications.

2130 Disruption of Bronchial Cell Monolayer Integrity by Organomodified Nanoclays and Their Incinerated Byproducts

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Organomodified nanoclays (ONCs) represent one of the most used engineered nanomaterials (ENMs) as nanofiller in emerging advanced manufacturing strategies to produce a diverse number of polymer nanocomposites. Different organic quaternary ammonium coatings on these 2-dimensional montmorillonite nanoclays allow for their incorporation in novel or replacement technologies in thin-film, aerospace, automobile, consumer, and health care polymer nanocomposite applications. Compared with other ENMs, little information exists on risks to occupational pulmonary health along the ONC life cycle that encompass synthesis, handling, manipulation, and disposal. This study hypothesized that coating type, incineration status, and time-dependent effects of ONC exposure would impact bronchial epithelial cell monolayer integrity, a key target following inhalation exposure. High-throughput *in vitro* screening strategies including high content imaging, electric cell impedance sensing, and flow cytometry were employed to evaluate a

set of pre- and post-incinerated ONCs for acute effects and fate of the monolayer post-exposure. Using each particle's IC_{50} cell viability in a BEAS-2B cell model, pristine nanoclay exposure caused acute loss of monolayer integrity, decreased metabolism, and increased apoptosis. Three different ONCs, however, displayed minimal loss to monolayer integrity despite coating type-dependent differences in apoptosis induction and decreased cell metabolism. Conversely, incinerated nanoclay byproducts caused decreased monolayer integrity, increased cell necrosis, and little evidence for reestablishment of the epithelial monolayer. These results suggest the type of quaternary ammonium coating and incineration status largely impacts mechanism of cytotoxicity, cell metabolism, and the recovery ability of the exposed bronchial epithelial cell monolayer. An integrated high-throughput *in vitro* screening strategy, using high content imaging and traditional *in vitro* methods, represents a rapid pulmonary epithelial toxicity assessment approach to prioritize ENMs for further evaluation and serves to inform 'prevention-by-design' material development strategies.

PS 2131 Selective Uptake of Carboxylated Multiwalled Carbon Nanotubes via Class A Type 1 Scavenger Receptors Impairs Viability and Phagocytic Activity in Macrophages

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Class A type 1 scavenger receptors (SR-A1) are involved in a variety of immunological and pathological responses. Our published work suggested that SR-A1 may be involved in the marked accumulation by cells of carboxylated multi-walled carbon nanotubes (C-MWNTs) compared to pristine MWNTs (P-MWNTs) (Wang et. al., *Nanotoxicology*, 2018). The studies in this poster confirm and extend the role of SR-A1 in mediating C-MWNT uptake. 1. Selective C-MWNTs accumulation correlates with surface SR-A1 receptor expression. RAW 264.7 and wild type B6 macrophages, which express high SR-A1 levels, accumulated high amounts of C-MWNTs, but not P-MWNTs or amino-functionalized MWNTs (N-MWNTs) at 37 °C. However, ZK macrophages, which are genetically deficient in SR-A1 receptors, did not accumulate significant amounts of C-MWNTs above background level. Further, stably transfected CHO cells that express mouse SR-A1 receptors had increased uptake of C-MWNTs compared to untransfected controls. 2. C-MWNT accumulation impairs phagocytic function via SR-A1 receptors. The physiological impact of C-MWNTs on subsequent phagocytosis of ligands that are known to interact with SR-A1 receptors was determined using confocal fluorescence microscopy and flow cytometry. There were significant reductions in the uptake of polystyrene beads and *E. coli* by RAW 264.7 cells pre-treated with C-MWNTs, but not with P- or N-MWNTs. The study on oxLDL uptake is underway. Further, the 24h continuous exposure of RAW 264.7 cells to C-MWNTs reduced surface SR-A1 receptors by almost 50%. 3. C-MWNT accumulation impairs macrophage viability. The impact of C-MWNTs on the proliferation of RAW 264.7 cells was assessed using a crystal violet assay and an 8d colony formation efficiency (CFE) assay. The accumulation of C-MWNTs impaired cell proliferation with IC50 values of 120 and 80 µg/mL for 48 and 72h exposure, respectively. P- and N-MWNTs have minimal effect on cell proliferation. In CFE assays, C-MWNTs reduced colony formation during an 8 day exposure with and IC50 of 29 µg/mL. These results confirmed that SR-A1 significantly contributes to the selective uptake of C-MWNTs and that SR-A1-mediated uptake of C-MWNTs impaired SR-A1-dependent phagocytic activities in macrophages and reduced cell viability.

PS 2132 Effect of Titanium Dioxide Nanoparticles on DNA Methylation in Human Cells

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The dramatically increased use of nanomaterials, including nanoscale titanium dioxide (TiO_2), raised the concern of their potential risk to human health. The present study aimed to further the understanding of the underlying mechanism of TiO_2 nanoparticles toxicity by examining the time-dependent alterations in cytosine DNA methylation induced by TiO_2 nanoparticles exposure *in vitro*. Human skin (A-431), lung (NL20), liver (HepG2), and colon (Caco-2) cells were treated with TiO_2 nanoparticles at the sub-cytotoxic doses for 24 and 72 hours. Treatment with TiO_2 nanoparticles resulted in global and gene-specific cytosine DNA methylation alterations. In particular, global DNA methylation decreased in three cell lines (Caco-2, HepG2, and A-431), while across the four examined cell lines, eight genes indicative of cellular stress response and toxicity (CDKN1A, DNAJC15, GADD45A, GDF15, INSIG1, SCARA3, TP53, and BNIP3) exhibited increased DNA methylation. Additionally, treatment with TiO_2 nanoparticles altered the expression of genes involved in establishing and maintaining DNA methylation patterns (DNMT1, DNMT3A, DNMT3B, MBD2, and UHRF) in cell-type- and time-dependent manner, with the greatest effects being found in NL20 and A-431 cells. The results of this study demonstrate that the sub-cytotoxic concentrations of TiO_2 nanoparticles induced treatment-related cytosine DNA methylation changes, indicating the potential value of epigenetic evaluation in the toxicity assessment of nanoparticles.

PS 2133 Survival Mechanisms in Keratinocytes Exposed to Subtoxic Concentrations of Metal-Derived Nanoparticles and Their Susceptibility to Xenobiotic Exposure

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Metal-derived nanoparticles (Mt-NPs) are increasingly used in cosmetology due to their ultraviolet shielding (titanium dioxide [TiO_2]), antioxidant (cerium dioxide [CeO_2]), and/or biocidal (silver [Ag]) properties. In the absence of overt toxicity (i.e. cell death), Mt-NPs are considered safe for their use in cosmetology. However, there is little understanding about the mechanisms involved in the survival of keratinocytes exposed to subtoxic levels of Mt-NPs, an issue that we aimed to address in this study. Human keratinocytes (HACAT) were exposed subacutely (48-72 h) to subtoxic concentrations (≤30 µg/ml), of rutile (r) TiO_2 (cylindrical), CeO_2 (cubic) and Ag (spherical) with a core / hydrodynamic size of <40 / <100 nm and >98% purity. Mt-NP uptake was quantified by changes in the light side scatter (SSC), where the kinetics (time-dose-response) suggested that they were internalized to a similarly extent by keratinocytes. rTiO_2 and CeO_2, but not Ag NPs, increased autophagy, and inhibition of autophagy flux (chloroquine) and autophagosome elongation (3-methyladenine) prompted cell death. In contrast, no increase in the steady-state levels of reactive oxygen species (ROS) was induced by exposure to any of the Mt-NPs tested. Interestingly, intracellular Ag aggregates observed a far-red autofluorescence (≥740 nm em), which has been ascribed to their binding to thiol molecules such as glutathione (GSH). Accordingly, inhibition of GSH synthesis with buthionine sulfoximine sensitized keratinocytes to Ag, but 6-aminonicotinamide, which impairs the recycling of oxidized GSH, had no effect on cell viability. rTiO_2 and Ag, compromised metabolic flux (glycolysis and mitochondrial respiration), but ATP levels were unaltered. Finally, we observed that Mt-NPs sensitized keratinocytes to non-UV xenobiotics (arsenite and paraquat). Our results demonstrate the differential contribution of autophagy and GSH metabolism to the survival of keratinocytes exposed to subtoxic concentrations of Mt-NPs, and highlight the increased susceptibility of keratinocytes exposed to Mt-NPs to xenobiotic exposure.

PS 2134 The ToxTracker Reporter Assay as a Tool for Mechanism-Based (Geno)toxicity Screening of Nanoparticles: Metals, Oxides, and Quantum Dots

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The increased manufacturing and use of nanoparticles (NPs) require faster and more efficient testing of their potential toxic effects. Traditional *in vitro* genotoxicity assays are often time-consuming and have the limitation that they generally lack the ability to provide insight into the mechanisms. One alternative approach is the use of reporter cell lines to quickly assess the cellular stress response pathways that are activated after exposure to NPs. In this study the applicability of the ToxTracker reporter cell lines to identify the (geno)toxicity of various metallic- or metal oxide NPs (n=18) including quantum dots (QDs) of various sizes was explored. The ToxTracker results were compared with historical genotoxicity results and physico-chemical characterization of the various nanomaterials. The results show a large variation in cytotoxicity of the tested NPs. Furthermore, the primarily effect observed in ToxTracker was activation of the oxidative stress reporters although some NPs also induced the reporters for DNA damage. Some NPs were non-cytotoxic and did not cause a clear activation of any of the reporter cell lines (Au, Cr, Cr_2O_3, Pt, SnO_2 and V). Other NPs were highly toxic e.g. antimony (Sb) NPs causing cytotoxicity and activation of reporters at relatively low doses (<2µg/ml). These effects were also observed for Sb_2O_3 but at much higher doses. NPs of manganese (Mn and Mn_3O_4) induced the most remarkable response in the ToxTracker assay with induction of reporters for oxidative stress, DNA damage, protein unfolding and p53-related stress. The CdTe QDs were also highly toxic showing clearly size-dependent effects and calculations suggest that surface area is the most relevant dose metric. Of all metal- and metal oxide NPs investigated (n=33), CuO, Co, CoO, CdTe QDs, Mn, Mn_3O_4, V_2O_5, and welding NPs clearly induced the Rtkn DNA damage reporter (> 2-fold). Also, NPs of Ni, NiO and Cr_2O_3 induced a weak response of this reporter (>1.5

fold, <2-fold). Thus, these NPs may be of particular concern when considering genotoxicity of metal- and metal oxide NPs. Furthermore, these results indicate that ToxTracker can be used for rapid screening, ranking and first indication regarding which nanoparticles may be grouped together during risk assessment.

PS 2135 Assessing the Biological Impact of PEGylated Graphene Quantum Dots in THP1 Human Macrophages

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Novel uses of engineered nanomaterials in medical applications have increased patient exposures raising safety concerns that require further studies to elucidate cellular interactions at the nano-bio interface. Past evidence suggests that polyethylene glycol-functionalized (PEGylated) nanomaterials are largely biocompatible and elicit minimal immune/inflammatory responses compared to their uncoated counterparts. This finding was recently found to be contradictory in the case of graphene-related nanomaterials. Graphene quantum dots (GQDs) are a graphene derivative that are being investigated for different biomedical applications given their unique optical properties and high biocompatibility. The goal of this study was to assess the potential *in vitro* immunomodulatory effects of PEGylated GQDs on the expression of pro-inflammatory cytokines and NLRP3 inflammasome components in human macrophages. The influence of the polymer molecular weight (2K, 5K Da) and surface functional groups (carboxyl, amino, bare) on THP1 human macrophages was assessed by evaluating cell viability, cell uptake of nanoparticles, cytokine secretion, and lysosomal stability. Cells were exposed to 15, 100, or 200 µg/mL GQDs for 24 and 48 hr. No significant cytotoxicity was observed for bare and carboxyl-PEG-coated GQDs at all concentrations and different PEG molecular weights. For amino-PEG-coated particles, a decrease of 40% in cell viability was observed at concentrations >100 µg/mL after 48 hr at the 2KDa molecular weight. Likewise, amino-PEG-coated GQDs induced the higher production of pro-inflammatory cytokines in a concentration-dependent manner. Further, confocal imaging of cellular structure indicated a higher uptake of amino-PEG-coated nanoparticles, as well as lysosome dysfunction. Induced caspase 1 and IL-1β production after exposure to this group suggests the involvement of NLRP3 inflammasome components in this process. More importantly, our results indicate that by controlling PEG coating features in GQDs, such as surface functionalities (i.e., carboxyl and amino) and molecular weight, immunological reactions can be mitigated or stimulated and thereby provide useful tools for a safe-by-design approach.

PS 2136 Risk Assessment of *Fusarium* Synthesized Silver Nanoparticles on a Dermal Cell Line A431

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Regardless of development of nanotechnology and nanomaterials, their potential risks are also increasing. Although there is huge development in the synthesis of silver nanoparticles (AgNPs) by various routes and their applications, a little information is available on the risk associated with their use. Hence, the present study was focused on *Fusarium* mediated synthesis of AgNPs, followed by their in depth characterization and *in vitro* toxicity. We screened six *Fusarium* species viz., *F. graminearum*, *F. oxysporum*, *F. culmorum*, *F. tricinctum*, *F. equiseti* and *F. moniliforme* for the synthesis of AgNPs. The fungal filtrate of all these species shown the ability to reduce the aqueous silver ions into AgNPs. Analysis of all samples via UV-Visible spectrophotometry shown the absorption peaks at about 420 nm, which is specific for plasmon band of AgNPs. The Fourier Transform Infrared spectroscopic analyses indicated that proteins or peptides might have coated over the AgNPs, thereby avoiding their aggregation and therefore increasing their stability. This protein capping of nanoparticles provided AgNP a higher zeta potential value which was confirmed by Zeta Potential measurement. Nanoparticle Tracking Analysis (NTA) shown their mean diameter ranging from 25- 70 nm in aqueous media, whereas Transmission Electron Microscopic observation shown their size from 5-25 nm. *F. oxysporum* synthesised AgNPs were highly stable and uniform in size, hence, used for toxicity assessment. *In vitro* toxicity study was performed on A431 cell line derived from squamous cell carcinoma. The MTT assay concluded the IC50 value for AgNPs to be 32 µg/ml. The Lactate Dehydrogenase (LDH) leakage assay results demonstrated that exposure to AgNPs for 24 hours resulted in significant increase in LDH leakage in dose-dependent manner. Generation of Reactive Oxygen Species in cell exposed to AgNPs were confirmed by flow cytometry analysis. DNA fragmentation assay results showed AgNPs induced DNA damage to cells. The cell cycle analysis confirmed the higher percentage of cell arrest in G0/G1 phase due to cells treated with AgNPs exposure which supports the DNA fragmentation data. The study thus concludes that AgNPs cause damage to A431 cells, resulting into accumulation of ROS, DNA damage and injury to cell membrane. This mechanism of causing injury leads to death of cells exposed to fungal synthesized AgNPs. The data of the present study can be useful for safer application AgNPs.

PS 2137 Cytotoxicity of Zinc Oxide (Nano) Particles in a Rat Intestinal Cell Model: Effect on Cellular Glutathione and Mitochondria

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Zinc oxide nanoparticles (NPs) have a variety of commercial and biomedical applications ranging from UV filtration in sunscreen to anti-cancer, fungal and anti-microbial agents. Human oral exposure to zinc oxide NPs may occur following accidental or intentional ingestion, hand-to-mouth activity, or mucociliary transport following inhalation. This study assessed the cytotoxicity of two different sized ZnO particles (10 and 150 nm) in rat intestinal cells (IEC-6). The 10 nm particle is classified as a nanoparticle. Previously we have reported that the cytotoxicity of these two particles and Zn ions are time- and dose-dependent in these cells. Dissolution of the particles and $ZnSO_4$ in media with 10% serum protein was assessed over 24 h. Zn concentration in incubation filtrate was determined by ICP-OES and was assumed to be free Zn ion. Intracellular glutathione (GSH) concentrations and mitochondrial area were assessed in the IEC-6 cells using spectroscopic methods after a 4-h exposure to the particles (0.1 - 100 ug/mL) or $ZnSO_4$ (100 ug/mL). Dissolution studies in media showed that both particles formed soluble Zn ions, and that media components bind these ions. At 24 h, approximately 40% of $ZnSO_4$, 40% of 10 nm ZnO and 30% of 150 nm ZnO was detected as free Zn ion in the filtrate. In H_2O, 100% $ZnSO_4$ was detected in filtrate at 24 h, showing complete dissolution. A significant dose-dependent decrease in cellular GSH and mitochondrial area was detected in the IEC-6 cells after a 4-h exposure to both particles and $ZnSO_4$. Both cellular GSH and mitochondrial area in treated cells decreased up to 40% relative to non-treated cells. The results suggest that ZnO particles, of both the measured sizes, form Zn ions in media. Decreased cellular GSH may result from the reaction of this thiol with reactive oxygen species, which may be formed by Zn ions. The increased oxidative stress in the treated cells may also be damaging to the mitochondria. An imbalance of a cellular antioxidant such as GSH caused indirectly by Zn ions formed from ZnO particles may result in impairment of organelles such as the mitochondria resulting in cell death. *This abstract does not represent US EPA policy.*

PS 2138 Characterization of the Trajectory of Human Neural Progenitor Cells *In Vitro*: System Comparisons for Sex-Specific Developmental Neurotoxicity Testing

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Growing efforts have been designated to develop *in vitro* models capable of evaluating chemical effects on developing brains due to the relevance of this organ on the quality of human life. Several methods based on brain-derived cells in culture have been proposed; however, they were not designed to evaluate effects including biological variables such as sex. Sex influences cellular function and metabolism that may lead cells to respond to environmental toxicants in sex-specific manner, which, in turn, may result in difference of disease incidences between men and women. Our previous study has characterized the normal temporal pathway dynamics of differentiation of a promising *in vitro* model for toxicity screening by culturing human neural progenitor cells (hNPCs) of female origin (H9, hNP1™). In the present study, we have described the behavior of hNPCs originated from male donor (H14, NSC-H14) to verify the applicability of this hNPC line together with H9 in *in vitro* sex-specific developmental neurotoxicity testing. To compare, both cell lines were maintained under the same culture conditions (*i.e.*, coated plates, culture media, passage number) and their *in vitro* behavior were evaluated regarding doubling time, morphology, and dynamics of neuronal differen-

tiation (hematoxylin and immunohistochemical staining, and western blot) up to 21 days *in vitro* (DIV). Both hNPCs presented population doubling time of 72 hours and they were able to differentiate *in vitro* and to form neurons, increasing extension of neurite outgrowth and neuronal foci formation across *in vitro* culture. Hematoxylin staining allowed for a comparison of foci development. Western blot analysis with specific neuronal markers was used to track the similarities between the differentiation profile. Our characterization of normal development of hNPCs from male and female origins *in vitro* and observation of differences and similarities would be an essential tool when interpreting toxicological effects and examining role of sex in neurodevelopmental toxicity.

PS 2139 Biotransformed Nanomaterial Surface Charge Influence on Human Liver Cell Line Toxicity

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Interest in silver nanoparticle transformation has grown due to the roles they play in maintaining the sterility of biomedical products, food storage bags, and personal care products. Silver nanoparticles are intentionally ingested in fruit juices where they act as clarifying agents and unintentionally ingested when they leech from food packaging. While the literature has extensively studied the adverse health outcomes of silver nanoparticles, these studies have been limited to engineered nanoparticles. There is an increasing need to investigate the toxicity of biologically transformed nanomaterials. Ionic silver nanoparticles elicit toxicological effects at high and low concentrations, however it is unclear if biotransformed nanoparticles will illicit the same responses. Here, we studied biotransformed silver nanoparticles of either positive, negative, or neutral charge. Each nanoparticle system was incubated in different physiologically-relevant environments: acidic stomach fluid, neutral blood serum, and basic surfactant fluid. Their associated effects were studied on human hepatoma cells, HepG2. Endpoints of viability, oxidative stress, and DNA and mitochondrial damage were measured. Our results indicate that the positive and negative nanoparticles biotransformed with acidic stomach fluid increased oxidative stress in the cells compared to other nanoparticles due to their increased ion production. Nanoparticles incubated in neutral blood serum produced a net negative surface charge due to protein corona formation on the surface. The nanoparticle protein corona complexes were seen to have a decrease in adverse effects due to the protective protein corona masking potentially disruptive mechanisms from occurring. The nanoparticles incubated in basic surfactant fluid had negligible effects compared to the other biotransformed nanoparticles. Additionally, surface charge influenced the uptake mechanism in which the biotransformed particles entered the cell, which was confirmed by hyperspectral imaging. Monitoring the toxicological effects of biotransformed nanoparticles along their product life cycle is necessary to accurately describe real life exposure scenarios.

PS 2140 Changes in Cellular and Secreted Lipids after Exposure to Single-Walled Carbon Nanotubes Have Impacts on Susceptibility to Influenza A Virus

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Nanoparticles (NP) are used in industry and consumer products; however, we are just beginning to mechanistically understand how exposure to NP impacts human health. Research has focused on pulmonary endpoints such as fibrosis, allergic-type reactions, and cancer, while the area of viral susceptibility remains less well explored. Work by our group has determined that exposure of human small airway epithelial cells (SAECs) to single-walled carbon nanotubes (SWNTs) increases host susceptibility to Influenza A virus (IAV) infection, as evidenced by increased viral titers and repressed anti-viral gene expression. We also demonstrated that this effect is only observed if SWNT exposure precedes IAV infection by 24 hours, suggesting that the cells are "primed" in a way which makes them more susceptible to infection. To elucidate perturbations that might act as priming mechanisms, we investigated how SWNTs modulate cellular and secreted lipids using untargeted lipidomics. We hypothesized that SWNTs change intracellular membrane and secreted lipids, resulting in an environment that is more conducive to supporting a productive IAV life cycle. For our approach, we exposed SAECs to 20 µg/mL SWNTs for 24 hrs and collected the cells and media for separate lipidomics analyses. Lipids were extracted per the Bligh-Dyer method, followed by LC-ESI-MS/MS analysis. We observed enrichment of several classes and species in both the cellular and secreted lipid landscapes. In cells, SWNT exposure resulted in significant enrichment of phosphatidylcholines (PC), phosphatidyethanolamines (PE), plasmenyl species, sphingomyelins (SM), and oxidized triglycerides (OxTG). In the secreted lipidome, SWNT exposure resulted in significant increases in PE, oxidized lysophosphatidylethanolamines (OxLPE), plasmenyl species, SM, ceramides (Cer), and OxTG. We speculate that these changes could "prime" the cells to be more susceptible to virus infections, perhaps by altering the host cell membrane to aid IAV in entry and egress. These observations demonstrate a novel role for SWNTs in perturbing both cellular and secreted lipid landscapes, with downstream impacts on susceptibility to infection. Future work will focus on impacts of increasing or decreasing lipid levels in cells, in the absence of SWNTs, and the subsequent effects on IAV infection in SAECs. These studies highlight the important role that lipid metabolism plays in host-pathogen interactions and targets these pathways in SWNT toxicity.

PS 2141 Using a Model Lysosome Membrane to Study Nanomaterial-Membrane Interactions That Cause Cytotoxicity

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The increased production of engineered nanomaterials (ENM) have enhanced the risk of human exposure. Many types of ENM are taken-up by phagocytic cells, such as alveolar macrophages, where the ENM accumulate in phagolysosomes. Accumulated ENM have been shown to cause a leakage of phagolysosomes membranes, resulting in phagolysosomal membrane permeability and the release of hydrolytic enzymes into the cytosol, causing inflammation and cytotoxicity. This suggests that ENM may interact directly with lipid membranes. We designed model lipid systems to study interactions between ENM and lipid membranes to assess ENM-lipid interaction. Liposomes (100 nm) were generated from lipids found in lysosomes: bis(monooleoylglycero) phosphate (BMP), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), in a molar ratio of 2:3:3. Tris-buffered saline (TBS, pH 7.4) and acetate-buffered saline (ABS, pH 4.5) were used to mimic the pH of early and late lysosomes. Liposome structure was evaluated using transmission electron microscopy; all preparations were 100 nm in diameter and unilamellar. Liposomes were exposed to either two types of titanium dioxide (TiO_2) ENM, nanospheres (TNS) or nanobelts (TNB), or zinc oxide (ZnO) nanospheres for 2 hours at 25, 100 or 200 µg/ml. Time-resolved fluorescence anisotropy, a measure of depolarized light, was performed using the fluorescence membrane probe di-4-ANEPPDHQ and a custom-built time-resolved fluorometer to measure changes in membrane fluidity (reported in cone angle, a measure of probe wobble). Liposomes (BMP:DOPC:DOPE) exposed to TNS (100 µg/ml) showed no difference in cone angle versus control in either buffer. However, TNB exposure produced significant decreases in cone angle in TBS at 200 µg/ml (36.42±0.31° to 32.84±0.33°) and in ABS at both 100 and 200 µg/ml (34.92±0.52° to 34.30±0.83° and 30.88±0.12°). Even though the surface area of TNS was greater than TNB the lack of effect supported that shape was important. ZnO ENM exposed liposomes composed of BMP:DOPC:DOPE showed no significant change in cone angle (100 µg/ml). However, BMP:DOPC:DOPS liposome had a significant decrease in cone angle (37.60±0.35° to 34.12±0.30°), indicating lipid headgroup can influence interactions. *Funding: NIH R01ES023209, 1F32ES027324, P20GM103546, P30GM103338 and J Murdock Charitable Trust.*

PS 2142 Novel Approach for Characterizing Exposure and Response to Engineered Nanomaterials in the Gut

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Despite the expanding number of applications for engineered nanoparticles (ENPs), human health concerns associated with ingested nanoparticles are poorly understood. In this study we utilized 3D human small intestinal tissue model to develop a physiologically relevant test system to assess toxicological profiles of ingestible nanomaterials. We examined barrier integrity and cytotoxicity of the human 3D intestinal tissue model following exposure (4 doses of each nanoparticle to Cupric (II) oxide (CuO) (50 nm in size), zinc oxide (ZnO, 35-45 nm in size)), and titanium oxide (TiO_2, 40 nm in size). To monitor reproducibility of the test method the nanoparticles were tested using 3-4 intestinal tissue lots. To determine the effect of the nanoparticles on the small intestinal tissues, we examined 1) barrier integrity (TEER), 2) tissue viability (MTT), and inflammatory response (ELISA assay). In the various experiments performed, the tissues were exposed to 40 ul of different doses of sonicated nanoparticles under rocking condition for 4 hr. After 4 hrs, dosed tissues were further cultured for overnight under static condition. Using IC_{15} (concentration that reduces barrier function or tissue viability by 15%) as a cut off, we

observed a dose response reduction of barrier integrity and tissue viability for CuO and ZnO. However, Titanium oxide did not induce toxicity for the concentrations tested. Furthermore, culture supernatants collected from at 24 hr of the culture period were also analyzed for inflammatory response and the result showed a dose dependent release of IL-8 for CuO and ZnO. Overall, the TEER measurement was a sensitive endpoint compared to the MTT tissue viability assay. In summary, the use SMI tissue model to examine the toxicity profile of ingested nanotoxicity will also enhance our understanding of nanoparticle-host cell interaction, improve dose/design, generate a physiologically relevant data set, and provide greater insight into *in vivo* responses.

PS 2143 Silver Nanoparticles (AgNP) Exposures on *In Vitro* Proliferating and Differentiating Human Neural Progenitor Cells (hNPCs): Importance of Windows of Susceptibility

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Silver nanoparticles (AgNPs) have been used increasingly in various consumer, medical, and commercial products for their unique antibacterial properties. Increasing number of studies, however, have raised concerns regarding exposures to AgNPs regarding their potential adverse impacts on humans, specifically for effects on brain development and function. This study was designed to examine the effects of gold-cored AgNPs on *in vitro* human neural progenitor cell (hNPC) cultures and evaluate dose-response related impacts to using commercially available hNPC H9 (hNP1™). Three types of AgNPs—20 nm AgCitrate, 110 nm AgCitrate, and 110 nm AgPVP—were used, and the effects of these particles by size and coating were evaluated on proliferating at day 1 or differentiating hNPCs at day 1 or 7. Exposures to AgNPs (0 – 50 µg/mL) demonstrated significant dose-dependent decreases in cell viability. We discovered that the differentiating hNPCs at day 7 received the least impact compared to proliferating and differentiating hNPCs at day 1. For 20 nm AgCitrate, differentiating hNPCs at day 1 were the most susceptible to AgNPs; however, proliferating hNPCs at day 1 were most vulnerable to larger AgNPs (110 nm AgCitrate and 110 nm AgPVP). Significant effects of particle coatings, sizes, and neurodevelopmental stages were observed for all particles. Our results suggested that the "window of susceptibility" for AgNPs is dependent on particle sizes; however, the impacts may be greater during the early developmental period than late. Together with other *in vitro* neurodevelopmental models, these offer approaches to assess both environmental and time-specific factors influencing neurodevelopmental susceptibility.

PS 2144 Titanate Nanosheets Cause Vacuolar ATPase-Dependent Apoptosis of Human Monocytes through Lysosomal Toxicity Not Related with Augmented Autophagy

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Titanate nanosheet (TiNS) is a promising 2D material with very thin structure. In our previous study, it had been found that TiNS exposure caused caspase-dependent apoptosis of human monocytes with formation of giant vacuoles, in which there were engulfed TiNSs. Therefore, the present study examined functions of lysosome and autophagy in human monocytes exposed to TiNS. $CD14^+$ monocytes were magnetically purified from PBMCs and cultured with TiNS or TiO_2-P25 nanoparticles at 10 µg/ml. TiNSs, having thin and diamond-like shape with about 20 and 30 nm diagonals, were synthesized from $Ti(O\text{-}i\text{-}Pr)_4$ and NEt_4OH by a method of liquid-phase synthesis. Both of TiNS and TiO_2 caused increase in lysosome at the early stage of culture assayed by flow cytometry, but TiNS-exposure monocytes finally showed five times greater lysosome level than TiO_2-exposed those. On the other hand, when mRNA expressions of autophagy-related genes functioning in the stages of nucleation, omegasome formation and elongation were examined by realtime RT-PCR, TiNS-exposed monocytes showed increases in expression of many kinds of genes including ATG101, Beclin-1, ATG9A, ATG3, ATG7, ATG12 in contrast to TiO_2 exposed those, indicating augmented autophagic machinery. As autophagy finish through fusion of autophagosome and lysosome, we examined which excessive generation of lysosomes or augmentation of autophagic machinery is attributed to apoptosis of monocytes exposed to TiNS. Bafilomycin A1, which suppresses vesicular acidification by inhibiting vacuolar ATPase (v-ATPase) in lysosomes, reduced formation of giant vacuoles and apoptosis of monocytes upon exposure to TiNS. In contrast, wortmannin, an inhibitor for PI3K which functions in autophagy, did not increase or decrease apoptosis of monocytes upon exposure to TiNS. In addition, bafilomycin A1 partially restored suppression in cell growth of human monocyte-like cell line THP1, whereas wortmannin caused more suppression in it. Those results indicate that TiNS has a harmful potential to cause v-ATPase-dependent apoptosis of monocytes through lysosomal toxicity, not related with augmented autophagy. Our findings suggest characteristic toxicity of TiNS which is not observed in TiO_2 nanoparticles.

PS 2145 Linking Aluminum Nanomaterial Induced Changes in Mitochondrial Ultrastructure to Alterations of Extracellular Flux: Structure/Function Validation of Mitochondrial Dysregulation

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The use of engineered aluminum nanomaterials as an additive in consumer and industrial products is increasing due to their special properties. Specifically, aluminum is highly utilized as a fuel additive, in automotive construction, and in a multitude of consumer products. There is a high risk of occupational, consumer, and environmental exposure, however the mitochondrial toxicity of aluminum and aluminum oxide nanomaterials is relativity unknown. Aluminum nanomaterials are known to accumulate within mitochondria and perturb mitochondrial processes, but the specific mechanisms of toxic action remain unknown. There is a need to develop high throughput toxicological testing methods focused on elucidating aluminum nanoparticle (AlNP) toxicities. The purpose of this study was to determine the extent of AlNP induced changes in mitochondrial structure and perturbation of mitochondrial health in an effort to link together these two endpoints. To determine changes in mitochondrial health, three different epithelial cell-types from the upper airway with varying phenotypes were selected as a test system. The three phenotypes include primary cells (PTBE), cancer cells (A549), and asthma cells (DHBE). These cells were selected because (1) they represent a wide range of cell lines that are commonly used in pulmonary toxicology and (2) it is possible to determine the extent the phenotype will modulate resultant toxicities. Mitochondrial ultrastructure was assessed via transmission electron microscopy while cellular mitochondrial biogenesis (i.e. ATP production) was measured using multiple assays on an extracellular flux analyzer. Differential dose-response patterns were seen in both the morphological and bioenergetic assessments. The alterations of mitochondrial shape and cristae structure integrity measured via transmission electron microscopy analysis was correlated to the resulting changes in mitochondrial coupling efficiencies as well as real-time ATP production. These results exemplify the necessity to conduct multitargeted analyses to better evaluate mitochondrial health. Furthermore, the techniques used to probe overall mitochondrial health and mitochondrial specific ATP production after exposure to environmental contaminants will become increasing important as the production of nanomaterials continues to increase.

PS 2146 Effects of Nanoparticle and Microbiota Exposure on Mucus Composition in an *In Vitro* Gastrointestinal (GI) Tract Model

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In vitro models of the gastrointestinal (GI) tract incorporating co-cultures of Caco-2/HT29-MTX-E12 have been shown to be a reliable and cost-effective experimental method to understand the effects of commonly used metal oxide nanoparticles (NP) as food additive. The relatively small size of NP enables them to easily cross cellular barriers and create free radical in tissues that can damage the cells and mucus layer that line the GI tract. The human GI tract is composed of a single layer of epithelial cells protected by a mucus layer, and the microbiota. The GI mucus forms a single, easily removable layer in the small intestine and a double layer firmly attached to the epithelium in the colon. The mucus traps and transports debris and bacteria, lubricates, and lowers mechanical stress on the epithelium. However, few studies have been performed on the intestinal mucus lining to better understand the role it plays as a protective barrier. The goal of this study is to determine if and how the mucus layer is affected by the presence of commensal, Gram positive (*LactoBacillus rhamnosus GG*) or opportunistic Gram negative (*Escherichia coli* ATCC 11775) bacteria and exposure to physiologically relevant doses of pristine or digested metal oxide NP using an *in vitro* model of GI tract. To observe and measure the role of bacteria on mucus function and how food additives might alter this relationship, cells monolayer was exposed to bacteria and physiologically relevant doses of TiO_2, ZnO, Fe_2O_3 or SiO_2 nanoparticles for up to four hours. Mucus was then stained with Alcian Blue (AB), Periodic Acid Schiff (PAS), or an Alexa Fluor® 488 conjugate of Wheat germ agglutinin (WGA) to determine mucus thickness and composition. The composition of mucosubstances in the cell culture model was then analyzed and the results indicate that mucus secretion increased in the presence of bacteria and sig-

nificantly decreased following exposure to pristine or digested NP and bacteria. Exposure to both commensal bacteria, opportunistic bacteria, and NP has impacts on the mucus layer. Changes in the distribution or pattern of acidic and neutral mucins are indicative of certain pathological conditions, and our model provides a platform for understanding changes in the mucus layer.

2147 High Content Single Cell Image Analysis of Sub-Cytotoxic Phenotypic Changes Driven by Carbon Nanomaterial Exposure

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Toxicologists experience challenges evaluating sub-cytotoxic toxicant exposures. We have developed a single-cell screening platform capable of evaluating high-content confocal images to quantify single-cell, sublethal phenotypic changes driven by carbon nanomaterial exposure. We hypothesized that dysregulation of fibroblasts, wound-healing cells responsible for excessive ECM deposition in fibrosis, could be driven in part by the mechanical stimuli associated with stiff, pristine carbon nanomaterials and that this response is masked by traditional stiff cell culture techniques. Through image segmentation pipelines and multivariable regression analyses, we identified single-cell features predictive of phenotypic change driven by carbon nanotubes and 5 µm graphene exposures, but not non-toxic carbon black or nano-graphenes. Soft substrate culture marginally decreases overall fibroblast differentiation but promotes significant changes to nuclear morphometry, cytoskeletal and nuclear texture, and differentiation mechanism endpoints. Cells exposed to any nanomaterial on stiff substrates have similar phenotypic signatures, while soft substrates sensitize cells to carbon nanotubes and group graphene exposures together, suggesting geometry-specific responses masked by stiff substrate culture. Multi-variable PCA plotting identifies a greater spread of soft substrate cell phenotypes associated with carbon nanotube and 5 µm graphene exposures, quantifying single-cell sensitivity to nanomaterial exposure typically unquantifiable without single-cell omics. Finally, we identified predictive phenotypic features through correlation and feature reduction analyses, highlighting 16 significant soft substrate feature relationships. These image analysis and quantification platforms can be used by toxicologists to identify predictive features of sub-cytotoxic and toxic effects in any cell type. Additionally, this universally relevant platform can also be used to evaluate the phenotypes of collected primary cells, stem cells, cancer-associated fibroblasts, etc. This work is currently being adapted to analyze monoculture and coculture 3D *in vitro* microtissue models of disease for nanomaterial toxicity screening. *This research is supported by the NIEHS Training Grant T32 ES07272, NIEHS Superfund Research Program P42 ES013660, NIEHS U01ES028184-02, and Unilever.*

2148 Understanding the Protective Role of Gut Microbiota following Metal Oxide Nanoparticles Exposure Used as Food Additives

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Nano-sized metal oxides are commonly added to processed foods and food packaging as have been demonstrated to improve food quality. The present study mainly focused on evaluating the potential effects of four different types of metal oxide nanoparticles (NP) that are commonly used in food packaging, like zinc oxide, or as food additives like iron oxide, silicon dioxide, and titanium dioxide. Since this NPs will be orally ingested, digested and finally reaching the duodenum (the first part of the small intestine), our laboratory has aimed to mimic this environment using an *in vitro* model of the gastrointestinal tract (GIT) called Caco-2/HT29-MTX. This model has gained attention in nanotoxicology as a useful method for evaluating the digestion of NP. The GIT environment, however, is composed of both human cells and gut microbiota. It is well-known that the intestinal microbiota, which is primarily composed of bacteria, forms a complex ecosystem and plays an important role in the health of the host. The gut microbiota has been shown to be involved in basic human biological processes, like regulating epithelial development and influencing innate immunity. Thus, to observe the role of bacteria in the gut function and to determine how NP can alter this relationship, we co-exposed the model with bacteria to all four NP and analyzed intestinal alkaline phosphatase (IAP), aminopeptidase-N (APN), sucrase-isomaltase (SI) and Na^+/K^+ ATPase activity. For that purpose, we used two common strains of commensal and opportunistic intestinal bacteria, *LactoBacillus rhamnosus GG and* a non-pathogenic *Escherichia coli* strain, respectively. Our results showed that following exposure to digested NP, the IAP, APN and SI activity was altered. Brush border enzymes are known to control many physiological gut processes and activities, such as the digestion of peptides and nutrient transport. Changes in their activity may be related to chronic diseases such as obesity, inflammatory processes, and malnutrition. However, the presence of bacteria attenuated the NP effects playing then a protective role toward the small intestine. We finally demonstrate by confocal imaging that these bacteria can attach, and in some cases surround, to big NP aggregates impeding them to get in contact with the cell membrane and cell microvilli.

2149 Elucidation of Carbon Nanodot Uptake Routes in Macrophages

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Carbon nanodots (CNDs) are among the most recent nanoparticles to generate interest in the research. CNDs are less than 10 nm in size and exhibit ideal and unique properties such as photoluminescence and biocompatibility. These properties make CNDs useful in bio-sensing, bio-imaging, and drug delivery. However, there is no report denoting potential uptake routes of CNDs into any cell type. In this study, THP-1 human monocytes were used to be differentiated into macrophages by 12-o-tetradecanoylphorbol acetate (TPA). We examined CNDs cellular uptake by quantification of the fluorescence of this nanoparticle in THP-1 monocyte-derived macrophages, and potential uptake routes by treatment with a selection of transport inhibitors. Cytochalasin A was used to inhibit the polymerization of actin and nocodazole was used to inhibit microtubule cytoskeleton. The result showed that CNDs were found to be uptaken into macrophages. However, cells treated with either cytochalasin A or nocodazole showed significant inhibition of CNDs uptake. These results suggest that both actin and microtubule cytoskeleton might be implied in the CNDs uptake mechanisms.

2150 Critical Evaluation of Potential Nickel-Containing Nanoparticles Toxicity

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The exceptional physical and chemical properties of nickel nanomaterials have been exploited in a range of applications including catalytic reactions, electrical conductors, magnetic materials, fuel cells, pigmentation, alloys, batteries, and biomedical materials. However, it has been suggested that these unique properties of nickel nanomaterials may allow for increased bioavailability, bio-reactivity, and potential adverse health effects. Therefore, the purpose of this study was to critically evaluate data regarding the toxicity of nickel nanoparticles with respect to: 1) physicochemistry - metal specification, surface chemistry, dissolution, size distribution, morphological characteristics, 2) quality of nanomaterial characterization in the defined delivery media, 3) routes of exposure and target organ effects, 4) appropriateness of model system and translation to potential human effects, and 5) current research data gaps and likely directions of future research. A literature search was conducted through October 2017 and a total of 112 relevant articles were identified. The reliability of the studies was evaluated using modified Klimisch scoring. Nickel oxide and metallic nickel were the most common forms of nickel nanoparticles and particle size ranged from 4 to 100 nm. Inhalation data in rodents indicated that the inflammatory response induced by doses of nickel nanoparticles up to 1222 µg/m^3 was characterized as acute in nature and only displayed chronic effects after relatively large exposures. Furthermore, there is no evidence, thus far, to suggest that the effects induced by nickel nanoparticles, including inflammation, oxidative stress, and cytotoxicity, are related to preneoplastic events. There is some data to suggest that nano- and micron-sized nickel particles follow a similar dose response when normalized to surface area. However, future experiments need to be conducted to better characterize the dose-response relationship according to surface area, which drives particle dissolution and potential biological responses. Taken together, this analysis critically evaluated the biological responses and associated health endpoints for nickel nanoparticles along with considerations that should be taken into account when interpreting the data.

2151 Activities of Renal and Testicular Electron Transport Chain Enzymes in Wistar Rats Exposed to Titanium Dioxide Nanoparticles

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Safety of Titanium dioxide nanoparticles (TiO_2 NPs) remains unclear because data on absorption, distribution, elimination or any adverse effects after oral exposure are extremely limited. Since disorders of energy metabolism are being recognized as incipient biochemical events in the toxicity associated with toxicants, this study determined the activities of electron transport chain (ETC) enzymes-Combined complex I+III, CPI+III; Combined Complex II+III, CPII+III; Complex IV, CPIV in male wistar rats exposed orally to TiO_2 NPs (8-12nm) (50, 150 and 250 mg/kg body weight [BW]) for 4, 8 and 12 weeks. Control rats received distilled water for the same period. At the end of TiO_2 NPs exposure, mitochondria were isolated from kidney and testes and activities of the ETC enzymes determined. While renal CPI+III was down-regulated at 8 and 12 weeks, renal CP II+III activity was increased at 4 weeks, with the highest increase occasioned by exposure to 150mg/kg BW dose of TiO_2NPs. At 12 weeks however, the three doses of TiO_2 NPs decreased the activity of the renal enzyme especially with the 250 mg/kg BW of TiO_2 NPs. Renal CPIV followed the same pattern as that of CPII+III. The three doses of TiO_2 NPs increased testicular CPI+III at 4 weeks, with the highest increase (5-fold increase) observed with the 150mg/kg BW of TiO2 NPs. While a duration-dependent increase in activity of testicular CPII+III was observed with exposure to 50mg/kg BW of TiO_2 NPs, a duration-dependent decrease was observed with the 250 mg/kg BW of TiO_2 NPs. The 150mg/kg body weight of TiO_2 NPs however increased the activity of the testicular enzyme by 60% at 4 weeks but decreased the same at 12 weeks by 26%. In contrast to CPII+III, testicular CPIV was characterized by a duration-dependent increase on exposure to the 250mg/kg BW and a duration-dependent decrease with the 150mg/kg BW dose of TiO_2 NPs. Our findings indicate that exposure to TiO_2 NPs modulates renal and testicular ETC and this might be important in renal and reproductive pathologies.

2152 Evaluation of *Hyalella azteca* Sensitivity to Acute and Chronic Silver Nanoparticle Exposures

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There is limited information on *Hyalella azteca* responses to silver nanoparticles despite the widespread incorporation of nanoparticles in medical and consumer products as a result of their antimicrobial properties. About 220 to 312 tons of AgNPs are produced globally per year and 0.1 ng/L AgNPs with 14 nm has been detected in surface waters indicating the presence of the nanoparticles in aquatic systems. *Hyalella azteca* are likely to be affected by AgNP exposure because they are highly sensitive to ionic silver. The current study evaluated AgNP toxicity to *H. azteca* in reformulated moderately hard reconstituted water to determine whether the amphipods are sensitive to nanoparticle exposure. Test organisms (7-8 days old) were exposed to different concentrations of 10 nm citrate-coated AgNP with five replicate beakers for 10 and 28 days in a static-water-renewal system. Ten *H. azteca* were added to each replicate beaker containing 100 mL sand as a substrate. Aliquots of test solutions were centrifuged, digested, and analyzed using furnace atomic absorption spectrometer to measure dissolved Ag, which was used for all statistical analyses. One-way ANOVA and Dunnett's test were used to determine the effects of citrate-coated AgNP on the survival and growth of *H. azteca*. Median lethal concentration (LC50) for survival and median effective concentration (EC50) for growth were calculated using probit analysis and Toxicity Relationship Analysis Trap respectively. Citrate-coated AgNP significantly reduced growth (dry weight) from 0.15 to 0.07 mg at 10.8 µg/L ($p < 0.05$) for the 10-day acute exposure. For the 28-day chronic exposure, citrate-coated AgNP significantly reduced growth from 0.63 to 0.40 mg at 7.2 µg/L ($p = 0.01$). The LC50 (95% confidence interval) for survival in the acute exposure was 7.8 (7.2-8.4) µg/L and the EC50 (95% confidence interval) for growth was 3.4 (1.2-9.4) µg/L. The LC50 for survival in the chronic exposure was 4.1 (1.4-5.5) µg/L and the EC50 for growth was 1.0 (0.1-8.3) µg/L. Growth was more sensitive to citrate-coated AgNP compared to survival in both acute and chronic exposures. We conclude for the first time that citrate-coated AgNP poses a potential risk to survival and growth of freshwater *H. azteca* populations, thus there may be the need to monitor the release of AgNPs into aquatic systems.

2153 Public Health Application of Sustainable Nano-Drugs and Nanocarriers

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Metal nanoparticles have found a vast range of applications in many commercial products, environmental remediation, and biological therapy. Some metal nanoparticles, particularly silver and copper, display antimicrobial properties at the nano scale. This concept further illuminates their potential to be synthesized and used in the control of disease pathogens of public health significance. In the present work, silver and copper nanoparticles were synthesized using three different capping agents and were characterized through dynamic light scattering (DLS), ultraviolet-visible spectroscopy (UV-Vis) and Fourier-transform infrared spectroscopy (FTIR). Silver and copper nanoparticles were synthesized and functionalized with surface capping agents to give them unique surface properties; polyvinylpyrrolidone (PVP), cetyltrimethylammonium bromide (CTAB), and citrate (Cit) coated silver nanoparticles as well as PVP, CTAB and ascorbic acid (AA) copper nanoparticles were synthesized. The synthesized nanoparticles were exposed to Gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* bacteria to assess antimicrobial sensitivity of the nanoparticles using the disk diffusion method. The results show that AA coated copper nanoparticles and PVP coated silver nanoparticles had the highest mean zones of inhibition against the growth of *E. coli* (11.56 mm, 10.44 mm respectively) and *S. aureus* (17.33 mm, 14.67 mm respectively) in comparison to other nanoparticles after 24 h of incubation. The Cit coated silver nanoparticles showed no effects on inhibition of both gram-negative and gram-positive bacteria growth. These results illustrate the efficacy of zerovalent nanoparticles as nanocarriers and nanodrug agents in the control of disease causing bacterial pathogens of public health significance due to their antibacterial properties at the nanoscale.

2154 A Novel Strategy to Evaluate the Degradation of Quantum Dots: Identification and Quantification of CdTe Quantum Dots and Corresponding Ionic Species by CZE-ICP-MS

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Understanding of the potential risks of quantum dots (QDs) to human health is becoming hotspot in the study of clinical applications of QDs. The gradation of QDs has been suggested to be the causes of their overall toxicity. However, further exploring the toxic mechanism of QDs still has a great technical challenge, especially how to identify and quantify QDs and its degradation products (corresponding ionic species) *in vivo* and *in vitro*. A method for the separation and simultaneous determination of CdTe QDs and its corresponding ionic species (Cd^{2+} and TeO_3^{2-}) in complex matrices was developed using capillary zone electrophoresis (CZE) coupled to inductively coupled plasma-mass spectrometry (ICP-MS) for the first time. Under optimal experiment conditions, the Cd^{2+} in different matrices was proportionally transformed into Cd^{2+}[1]and Cd^{2+}[2] species. The Cd^{2+}[1] was considered to be a kind of complex of $Cd^{2+}$and serum albumin. The Cd^{2+}[2] was probably a Cd^{2+}-$B_4O_7^{2-}$-H_3BO_3 complex. The values of the Cd^{2+} and Cd of CdTe QDs were quantified by the external calibrationcurve of Cd^{2+}[2] and Cd^{2+} (Cd^{2+}[1]+Cd^{2+}[2]), respectively, with a limit of detection(LOD) of sub-microgram per liter. The TeO_3^{2-} and Te of CdTe QDs were quantified by the external calibration curve of TeO_3^{2-} with a LOD of microgram per liter. Moreover, the quality assessment of commercial CdTe QDs and serum pharmacokinetics of synthesized CdTe QDs in rats were successfully undertaken by the developed CZEICP-MS. The identification and quantification of CdTe QDs and its degradation products can be determined simultaneously in a single run by the proposed CZE-ICPMS. This developed method provides a strategy not only for the systematic investigation of absorption, distribution, metabolism, excretion, and transformation (ADME/T) of QDs in animal tissue and cells but also for comprehensively understanding the degradation rules of QDs and their toxicity mechanism.

2155 Effects of Transport Inhibitors on the Cellular Uptake of Carbon Nanodots in Human Microvascular Endothelial Cells

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Carbon nanodots (CNDs) are a new form of nanoparticles that have been utilized in many areas in medical research. With these particles being less than 10nm in size and possessing unique photoluminescent characteristics, they

receive much attention for their potential usages in biosensing, bioimaging, and drug delivery. With the potential biomedical applications of CNDs, knowing and understanding their route of uptake into a cell is a critical piece of information that is still unanswered. In this study, we examined the possible route of uptake of CNDs within human microvascular endothelial cells (HMECs) by using several known transport inhibitors. The result showed that CNDs can uptake into HMECs, which utilize the intrinsic fluorescence of CNDs that has an emission signal at 460 nm upon excitation with a 360 nm laser. CNDs uptake was significantly affected by ebselen, which is a known inhibitor of mammalian H+, K+-ATPase, n-phenylanthranilic acid, a known chloride channel inhibitor, and chlorpromazine, a known suppressor of clathrin disassembly. Our data has suggested that CNDs might use different pathways to enter endothelial cells.

2156 ICONS: Integrated Testing Strategy for Mechanistically Assessing the Respiratory Toxicity of Functionalized MWCNT

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Toxicological concerns are opposed to the promising technical properties of multiwalled carbon nanotubes (MWCNT). Meeting WHO fiber criteria MWCNT can be carcinogenic (rigid type) or non-carcinogenic (tangled type) depending on fiber length and diameter. The project ICONS focused on the comparison of core MWCNT vs. surface-modified MWCNT (both tangled type) regarding their fibrotic and genotoxic potential. Purification of core MWCNT (chemically or thermally) and surface functionalization (-COOH or -NH2) of the industrially relevant Nanocyl NC7000 were varied. At Fraunhofer ITEM, the eight resulting MWCNT (pristine, milled, purified, and functionalized) were tested for sterility and endotoxin contamination. For *in vitro* use, they were dispersed using an ultrasound-based protocol, and characterized by light and scanning electron microscopy. Subsequent *in vitro* (geno)toxicity testing with MRC-5 primary human lung fibroblasts revealed differential inhibition of proliferation (RICC, mitotic index) and induction of membrane damage, DNA-strand breaks and micronuclei. Using primary human mesothelial LP9 cells, a variable number of differentially expressed genes was noted. Based on these *in vitro* data and the *in vivo* data, generated by LTAP and NCSU, the COOH-functionalized chemically purified (NC3151) and thermally purified (NC-PlacylCOOH) samples were selected for a 4-wk inhalation study in rats (design based on OECD TG 412; 0.2, 1 and 5 mg/m^3), including a 4-wk recovery (validation test). As a reference group, the thermally purified NX7100 sample (non-functionalised core) was included with 5 mg/m^3 only. Pre-trials demonstrated feasibility of generating respirable MWCNT aerosols by dry dispersion with pressurized air, supported by a jet mill. The differential cell count analysis, the levels of lactic dehydrogenase, beta-glucuronidase and total protein in bronchoalveolar lavage fluid and the histopathological examination resulted in the following ranking: NC3151 < NX-7100 (core) = NC-PlacylCOOH, indicating that the purification method seems to be important. *This ERA-NET SIINN project was funded by the German BMBF (FKZ: 03XP0063).*

2157 Investigating Barrier Capacity of Human Placenta to Foodborne TiO_2 Nanoparticles (E171) Using an *Ex Vivo* Perfusion Model and Ti Quantification in the Placenta and Meconium

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Food-grade titanium dioxide (TiO_2, white pigment E171 in EU) contains up to 55% (number-based) of nanoparticles (NPs). Despite low intestinal absorption, human dietary exposure is chronic (0.2-10 mg/kg/day) and TiO_2-NPs pass to the bloodstream and accumulate in systemic organs. In rodents, perinatal exposure to TiO_2-NP models showed transplacental transfer with health effects in offspring. The current study aims at evaluating whether the NP fraction of E171 may cross the human placenta. Term placentae and meconium were collected after delivery with mother consents. Basal titanium (Ti) levels were assessed by inductively coupled plasma-mass spectrometry (ICP-MS). Crystal TiO_2 forms were assessed on placenta tissue sections and fetal exudate by energy dispersive X-ray analysis (EDX) coupled with transmission and scanning electron microscopy (TEM/SEM-EDX), respectively. Placentae were perfused in a double open circuit for 30min of equilibrium with Earle's medium (EM), followed by 1h of EM alone (controls, n=2) or supplemented with E171 (15µg/ml, n=7). Passive antipyrine transfer rate served as viability marker. Passage of laser-reflective particles was evaluated by confocal microscopy on fetal exudate every 5min, and particle nature and size analyzed by SEM-EDX and ImageJ software. Basal Ti levels were 0.10±0.13 mg/kg in 92% of placentae (n=23/25), and 0.19±0.13 mg/kg in 36% of meconium (n=4/11). Anatase and rutile TiO_2 particles were commonly found in placentae. Laser-reflecting particles were detected in fetal exudate 10min after E171 addition in the maternal side. SEM-EDX imaging showed TiO_2 particles of diameters <200nm in the fetal side, 83% of them were NPs. In conclusion, circulating TiO_2 accumulates in the human placenta with Ti recovered in the meconium, demonstrating materno-fetal passage. *Ex vivo*, a placental transfer of E171 (TiO_2) particles mostly concerned NP fraction. These data emphasize the need of risk assessment in pregnant women of chronic exposure to TiO_2-NPs of dietary origin.

2158 DNA Damage Caused by Nickel Nanoparticle Exposure in Lung Epithelial Cells

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Nickel and nickel compounds are highly carcinogenic and nickel nanoparticles (Nano-Ni) are becoming increasingly used in industry (e.g. magnetic tape, pastes, catalytic materials, microfilters, etc). More interestingly, due to unique chemical and physical properties of nanostructure, nickel alloy nanomaterials have received special interest in biomedical applications. Our previous studies showed that exposure to Nano-Ni caused severe and persistent lung inflammation, which were strongly associated with pulmonary toxicity. In addition, exposure to Nano-Ni caused HIF-1α nuclear accumulation. The overall hypothesis of this study is that Nano-Ni may exert genotoxic effects via alternation of cell homeostasis through a mechanism mediated by activation of ataxia-telangiectasia mutated (ATM) and HIF-1α accumulation. We compared the ability of non-toxic doses of Nano-Ni and Nano-TiO_2 to cause DNA damage and explored the possible mechanisms. Our results showed that exposure of normal human bronchial epithelial cells BEAS-2B to Nano-Ni caused a dose- and time-response increase in the expression of phosphorylated histone H2AX (γ-H2AX), Rad51 and phosphorylated p53, indicating DNA damage. However, exposure to Nano-TiO_2 did not cause those effects. To investigate the potential pathways involved in the Nano-Ni-induced DNA damage, we determined the phosphorylation of ATM and found that phosphorylation of ATM was increased when BEAS-2B cells were exposed to Nano-Ni. Furthermore, pre-treatment with KU55933, a specific inhibitor of ATM, suppressed Nano-Ni-induced p53 phosphorylation and Rad51 expression. Our results also showed that exposure of BEAS-2B cells to Nano-Ni caused HIF-1α accumulation, up-regulation of miR-210, and down-regulation of Rad52. Rad52 is a key factor in homologous recombination repair. Our *in vivo* studies also showed that exposure of mice to Nano-Ni caused increased expression of phosphorylated γ-H2AX and Rad51, and enhanced phosphorylation of p53 in the lungs. Thus, Nano-Ni-induced DNA damage may be induced by Nano-Ni-induced ATM activation and HIF-1α accumulation. These findings have important implications for understanding the potential health effects of nanoparticle exposure. *This work was partly supported by ES023693, ES028911, and HL147856 from NIH, and Kentucky Lung Cancer Research Program to Dr. Qunwei Zhang.*

2159 Modeling the Influence of Carbon Nanotube and Nanofiber Physicochemical Properties on Key Molecular Initiating Events and Functional Endpoints Using Epithelial, Macrophage, and Fibroblast Cell Models

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There is a significant interest in using *in vitro* systems to evaluate toxicity of ever-increasing nanomaterial variants and other toxicants. We used 7 multiwalled carbon nanotubes and 2 carbon nanofibers (CNT/F) from U.S facilities to evaluate predictability, sensitivity, and the relationship of physicochemical characteristics to key molecular initiating events and functional responses following exposure. Additionally, the predictability of *in vitro* model systems to *in vivo* outcomes from a complementary study was determined. The CNT/F represent a good model as they are known to induce cytotoxicity, inflammation, pathology, and genotoxicity. The particulates had a wide distribution in

length (0.1-50 µm), diameter (6-397 nm), dustiness (0.2-4.9 %), metal contaminants (0.3-6.2 %), surface area (18–238 m2/g), and density (0.007–0.22 g/cm3). Endotoxin and PAH levels were below detection limit and zeta potential were similar for all materials. Genotoxicity was evaluated in human lung epithelial cell line, BEAS-2B, at 0-24 µg/ml. Acute toxicity, inflammation, inflammasome signaling, and phagocytic activity were evaluated in the differentiated human monocyte cell line, THP-1, at 0-60 µg/ml. Collagen production, TGFβ levels, and αSMA signaling were evaluated in primary human lung fibroblast cells at 0-9.6 µg/ml. Unsupervised approaches were initially used to identify classes of materials with similar outcomes followed by supervised learning approaches to identify specific physicochemical characteristics driving toxicity responses. It was clear certain physicochemical characteristics were the primary drivers of specific outcomes. Often, a multifactorial approach, meaning a combination of physicochemical characteristics, best described a particular outcome. Analysis of complementary endpoints in a concurrent *in vivo* study indicated some *in vitro* tests shared similar predictability suggesting some utility for predictive *in vitro* toxicity evaluation. These included specific measures of inflammation and pathological outcomes. The general conclusions of the analysis suggest that the class of materials, carbon nanotubes and nanofibers, can be subdivided based on specific endpoints, some aspects of *in vitro* outcomes predict *in vivo* toxicity, and the methodological approach can possibly be adapted beyond the finite scope of this study.

PS 2160 The Role of Macrophage Surface Receptors on the Uptake and Binding of Protein-Coated MWNTs

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Multi-walled carbon nanotubes (MWNTs) pose a human respiratory hazard because they can cause pulmonary fibrosis, which may lead to mesothelioma. A key event in the fibrotic pathway is the interaction of MWNTs with macrophages that may result in chronic inflammatory responses, but the mechanisms are not clear. An important question is whether macrophages have MWNT receptors that might initiate or modulate signals involved in inflammation. A complicating factor is that *in vivo* a protein corona, derived from serum proteins such as bovine serum albumin (BSA), may influence the interaction of MWNTs with cells. The interaction of BSA-coated carboxylated MWNTs (cMWNTs) and pristine (pMWNTs) with mouse RAW 264.7 macrophage cells was studied using a gel electrophoresis assay to quantify cell-associated MWNTs (Wang et al., *Anal. Chem.* 2009, 81, 8, 2944-2952). Both MWNT types accumulated as a function of time and concentration. To assess the potential role of cell surface receptors on macrophages, the binding of BSA-coated cMWNTs and pMWNTs to RAW 264.7 cells at 4°C in medium without serum was measured. At low temperature phagocytosis is inhibited, so that only binding of MWNTs on the cell surface is measured. Further, the absence of serum eliminates complications in interpreting the data that could arise due to the interaction of other serum proteins with the MWNTs to form an undefined protein corona. These studies directly demonstrated binding of both MWNT types to the cell surface that was a saturable function of MWNT concentration, supporting the idea that receptors bind BSA-coated MWNTs. The effect of BSA on the binding of BSA-coated MWNTs to the cells at 4°C showed that BSA reduced binding by 50%. Previous work suggests that scavenger receptors on macrophages bind cMWNTs (Wang et. al., Nanotoxicology, 2018, 12, 7, 677-698, DOI: 10.1080/17435390.2018.1472309). We found here that dextran sulfate, a known antagonist of Class A scavenger receptors, inhibited the binding of BSA-coated MWNTs to RAW 264.7 cells by a maximum of 50%. This suggests that on these macrophages there might be dextran sulfate sensitive and insensitive receptors.

PS 2161 Toxic Effects of Molybdenum Trioxide Nanoparticles (MoO_3 NPs) on Rat Pleural Mesothelial Cells (RPMCs) and Lavaged Cells from the Lungs of Golden Syrian Hamsters

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MoO_3 NPs are used in industrial, agricultural and biomedical applications due to their physical characteristics. The risk of exposure and possible adverse health effects to humans increases during the manufacturing and handling of such materials. The purpose of this study was to investigate the toxicity of MoO_3 NPs in cultured cells and in the cells isolated from bronchoalveolar lavage fluid (BALF) from Golden Syrian hamsters. Cultures of RPMCs (CCL-216) were exposed to MoO_3 NPs (100, 200, 300, 400, 500, 600, 700 or 800µg/mL) for 24 hours and compared to a vehicle control. Cytotoxicity was assessed by MTT and LDH assays. A concentration of 400µg/mL MoO_3 NPs was further evaluated (~LC_{50}). Capsase 1 and 3 protein levels were also measured in both groups. In addition, hamsters were exposed via inhalation and divided into four groups: 1) no exposure, 2) exposure to aerosolized water for 4h/day for 8 days, 3) exposure to 5mg/m^3 MoO_3 NPs for 4h/day for 8 days (*5-NP*) and 4) a group given a recovery period of one week (*5-REC*) following a *5-NP* exposure. Cultures of RPMCs treated with MoO_3 NPs had increased levels of: LDH (178%), caspase 3 (14%) and caspase 1 (29%) as compared to a control. BALF from the *5-NP* group had increased: total protein levels (62%), total cell counts (58%), neutrophils (870%) and multinucleated macrophages (465%) as compared to controls. BALF from the *5-REC* group had displayed parameters similar to controls, but had an increase of lymphocytes (938%) compared with controls. Tissue sections from the lungs of MoO_3 NPs treated groups of hamsters had membrane blebbing and hyperplasia of the airway epithelia with areas of cell proliferation at the periphery of the lung. Hamsters of the *5-NP* group had increased TUNEL positive cells in airway epithelia. Results from this study indicate cytotoxicity in RPMCs treated with 400µg/mL MoO_3 NPs may be mediated by both oncosis and apoptotic pathways. Animals exposed to 5mg/m^3 MoO_3 NPs had an initial acute inflammatory reaction as indicated by an alteration of BALF cells and protein levels that resolved to a chronic reaction with increased lymphocytes and hyperplasia in the airways. Cellular membrane blebbing in airway epithelia and an increase in TUNEL positive cells in hamsters exposed in the *5-NP* group support apoptosis as a possible pathway of injury.

PS 2162 *In Vivo* Lung Toxicity Associated with Boron Nitride Nanotubes with Different Purities

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Boron nitride nanotubes (BNNTs), a newly emerging nanomaterial with enhanced physicochemical properties, are increasingly incorporated into industrially relevant applications. Currently, commercial production of BNNTs is synthesized with 30-60 % residual compounds and impurities. The goal of this study was to assess the lung toxicity associated with *in vivo* exposure to BNNTs with various purities. Three BNNT samples with a gradient of purity were provided by the National Research Council, Canada, to assess lung toxicity *in vivo*: a low purity as-produced sample with ~50% BNNT (BNLP), an intermediate purity sample (BNMP), and a highly purified sample with >90% BNNT (BNHP). Hexagonal boron nitride (h-BN, <100 nm in diameter) was used as a control material for one of the by-products during synthesis affecting the purity of the BNNT material. All BNNT samples tested were shown to be agglomerated bundles of BNNTs (~3 to 5 walls/tube) with boron and h-BN as the primary impurities. Sample purity was confirmed by electron microscopy (EM). The BNNT samples prepared in dispersion medium (DM) were 0.5-1.5 µm in length and 5-30 nm in diameter. Male C57BL/6 mice were exposed by oropharyngeal aspiration to 4 or 40 µg of sample/mouse dispersed in DM or DM alone on day 0. Animals were euthanized at 4 h, 1 d, 7 d, 1 m, and 3 m post-exposure and lung lavage was performed to evaluate lung injury and inflammation. At 4 h post-exposure, lactate dehydrogenase (LDH) activity and neutrophils influx, indicators of lung injury and inflammation, were significantly increased by high dose of BNMP and BNHP. At 1 d and 7 d post-exposure, the effects were greatest in the high dose of all three tested BNNT samples, with BNHP>BNMP>BNLP, and persisted in the BNHP group up to 1 m post-exposure. Irritant response, indicated by eosinophils increase, was observed in the high dose of BNNT groups at 1 d and 7 d post-exposure, with BNHP>BNMP>BNLP. Lung lymphocytes continued to increase in the BNHP group up to 1 m post-exposure. The results indicated that the tested BNNT samples induced acute toxicity and inflammation only at high concentration and the effects were more pronounced with increasing purity. The reference material used to represent one of the by-products of synthesis, h-BN, did not show significant lung toxicity, suggesting lung effects where present may be due primarily to BNNTs.

PS 2163 Analysis of Nanoparticle Uptake by Disease Vector of Economic Importance

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The spread of vector-borne pathogens by arthropod hosts continue to cause human, animal and plant diseases of public health and economic importance. Using the integrated vector management approach, this study utilizes nanoparticles as potential targeted anti-pathogenic agents for the control of citrus greening disease. *Candidatus Liberibacter asiaticus*, the bacterial pathogen of citrus greening disease is spread by the Asian citrus psyllid (*Diaphorina citri Kuwayama*). Symptoms of the disease include blotchy mottle, yellow shoots, and improperly developed fruits. The significant decrease in edible

fruit production caused by the disease has led to decreased productivity of citrus farms which in turn causes negative significant economic loss to the citrus industry. Silver nanoparticles were synthesized, and surface functionalized with charged and uncharged groups. The resulting nanoparticles were characterized for size and shape measurements using dynamic light scattering (DLS), transmission electron microscopy (TEM) and atomic force microscopy (AFM). Ultraviolet-visible spectroscopy (UV-Vis) of nanoparticles was done to characterize the differences in surface coating of the nanoparticles followed by Fourier-transform infrared (FTIR) analyses for functional group characterization. Exposure studies carried out on the psyllid vector show differential accumulation of silver nanoparticles based on surface coating. Inductively coupled plasma mass spectrometry (ICPMS) show that negatively charged silver nanoparticles coated with citrate had the highest accumulation concentration (8.27 µg/L) after 96 h of exposure through an artificial feeding media compared to positively charged and uncharged silver nanoparticles. The result obtained from this study are indicative that other arthropods would have similar responses to nanoparticle exposure and that this approach of disease control may be translatable to other vectors of pathogenic diseases.

2164 An In-Depth Multi-'omics Investigation on Different Nanomaterials: A Promising Tool to Support Nanomaterial Grouping

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Nanomaterials (NMs) can be manufactured in plenty of variants demanding for alternative approaches for hazard assessment such as grouping and read-across. Multi-omics analyses are useful for establishing grouping and to justify read-across as they provide detailed insights into NM mode of action (MoA). Here we applied a multi-omics approach considering metabolomics, proteomics and transcriptomics for 12 different NMs including seven SiO_2 variants with a systematic variation of particle size, structure, surface charge and hydrophobicity as well as five other NMs, i.e. two organic pigments, TiO_2 NM-105, Graphene oxide and Mn_2O_3. All NMs were extensively characterized with respect to their physico-chemical properties. Toxicity was assessed *in vitro* in two different rat lung cell models, RLE-6TN rat lung epithelial cells and NR8383 rat alveolar macrophages, using several well-established assays. In addition, selected NMs were investigated *in vivo* in rats using short-term inhalation and instillation studies. Moreover, a multi-omics approach including proteomics, metabolomics and transcriptomics was applied for *in vitro* and *in vivo* samples. Our results demonstrate that NMs can be easily categorized based on changes in the profiles of the individual omics layers but a higher confidence was achieved in an integrated approach. Overall categorization was quite similar in both cell models and largely consistent with categorization based on well-established toxicity data. However, NM MoA was strongly cell-type specific. Overall, our approach appears to be very useful for NM grouping and we provide first examples how to integrate information on NM MoA.

2165 Do Impurities in Boron Nitride Nanotube Material Influence Toxicity?

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Boron nitride nanotubes (BNNTs) have applications in a wide array of industries. Prior BNNT toxicity findings vary and could be attributed to the many manufacturing processes by which BNNTs are produced. These manufacturing processes result in BNNTs with a divergent array of physicochemical characteristics, but commercial processes share a common challenge of having 30-60 % residuals and impurities. Here we evaluated the impact of these impurities on the toxicological profile of BNNT materials. Four BNNT samples made by induction thermal plasma process with a gradient of BNNT purity levels (~50-~90% pure) were used to assess toxicity. Hexagonal boron nitride (h-BN) (~100 nm in diameter) was used as a reference material. Electron micrographs confirmed a decrease in impurities and an increase in tubular structures across the gradient. The BNNTs dispersed in dispersion media decreased in as-produced dimensions and had a length of ~0.5-1.5 µm, diameter of ~5-30 nm, hydrodynamic diameter of 284-396 nm, surface area of 23-150 m^2/g and density of 0.01-0.27 g/cm^3. Electron paramagnetic resonance showed no change in acellular oxidative stress potential among the BNNTs of various purities. Cytotoxicity, proliferation, cellular oxidative stress, NF-κB-based induction of inflammation, and inflammasome activation were assessed in differentiated human monocytes (THP-1) at a concentration range of 0-100 µg/mL. There was a small increase in cytotoxicity and membrane damage with the highly purified BNNT materials, which decreased as the purity of the mixtures decreased. Oxidative stress measured by 4-hydroxynonenal expression, NF-κB by NF-κB-SEAP-expressing THP-1 cells, inflammasome activation by cellular caspase-1 and supernatants for IL1β and IL18 showed a dose-dependent increase with increasing BNNT purity. At all measured end points there was minimal effect with h-BN at the measured concentrations. Computational modeling was used to identify the physicochemical characteristics that altered biological response. This work shows that BNNT mixtures manufactured through the plasma process have low toxicity in general; the increase in toxicity with increasing purity shows that the impurities have minimal role towards the toxicity endpoints measured.

2166 Characterization of Nanoparticle Transformations in Physiologically Relevant Fluids

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Nanoparticles are increasingly used in biomedical applications due to their high surface area to volume ratio, ease of surface functionalization, and inherent ability to be excreted. Metal nanoparticles of like surface charge have been shown to display repulsive tendencies; but at physiologically relevant pH, these same particles form a double layer enabling particle stability. When nanomaterials travel through the body to their target site, they encounter many different aqueous environments which may alter colloidal stability. We hypothesize that nanoparticles will become increasingly unstable as the physiologically-relevant fluid they encounter becomes increasing complex. Specifically, the stability of the nanoparticles will be directly related to (1) pH, (2) the concentration of proteins in the biofluid, and (3) the ionic strength of the surrounding matrix. To test this hypothesis, we compared three different surface-functionalized silver nanoparticles that result in positive, negative, and neutral surface charge, which was confirmed through electron microscopy and spectroscopic techniques. Each particle system was incubated in different physiologically-relevant environments: acidic stomach fluid, neutral blood serum, and basic surfactant fluid. Negatively charged particles were noted to undergo the most significant transformations, whereas the particles with neutral surface charges were seen to have the least transformation. The information obtained from these studies will provide crucial insight into colloidal stability of biotransformed nanoparticles, provide read-across comparisons between metal-based and polymeric-based nanoparticles, and aid in filling the literature gap in nanoparticle colloidal stability and how it affects nanoparticle biotransformation.

2167 Polymer Assisted *In Situ* Synthesis of Silver Nanoparticles with Epigallocatechin Gallate (EGCG) Impregnated Wound Patch Potentiate-Controlled Inflammatory Responses for Brisk Wound Healing in Both Normal and Diabetic Condition

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Sponsor: K. Ansari

Diabetic patients often come up with complications such as delayed skin wound healing due to markedly enhanced inflammatory phase. Similarly, non-diabetic patients may also suffer from chronic wounds without proper dressing which fails in maintaining the cycle of inflammation, proliferation, and remodeling of skin wounds. Although various clinically proven dressing materials are available, yet the scope of developing better efficacious materials, will help the patient's centric medicare. Embarking on the same philosophy, we have designed hydrogel wound patches based on biopolymer, Guar gum, and the proven microbicidal silver NPs to promote the wound environment for accelerated wound healing. Incidentally, modified polymer begets *in situ* synthesis of silver NPs of 6 to 12nm size, besides lending ionic surface

charges and impregnated antioxidant epigallocatechin gallate (EGCG) favors a controlled inflammatory response. The wound patches possess apt tensile strength, porosity, and swellability for absorbing wound exudates. Further, *in vitro* studies endorsed them as non-cytotoxic towards the skin Keratinocytes MSC-P5 and the post agent effect following exposure to the patch showed an unbiased response to *E. coli* K12 and *B. subtilis*. High-fat diet (HFD) induced C57 BL-6 diabetic mouse model along with Wistar rats for non-diabetic group was opted. Sub-cutaneous wounds were created and time dependent (3,6,9,12 and 15 days) wound closure validates its accelerated healing through better wound contraction (more than 3 folds compared to control), enriched cell proliferation around wound area by enhanced expression of KI 67, more blood vessels formation, promoted collagen deposition and enhanced vascularisation compared to commercially available wound dressing material (skin graft) Neuskin-F®. The synthesized formulation showed time dependent enhancement in VEGF, a key regulator in promoting re-epithelialization and suppression in key inflammatory cytokines IL-6 and IL-10. Toxicity assessment were also performed in terms of hematological, liver, and kidney functional parameters signifying the non- toxic nature of hydrogel wound patches.

2168 Acute Toxicity of Perfluorinated Alkyl Substances toward Mahi Mahi (*Coryphaena hippurus*) Embryos

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Perfluoroalky substances (PFASs) are persistent organic contaminants that have been detected in wildlife, humans and the environment. They have been associated with diverse negative health effects including cancers, neurotoxicity, thyroid malfunction, reproductive problems, immunotoxicity, lung toxicity, and hepatotoxicity. Previous studies have demonstrated toxicity PFAS in the zebrafish embryo, as a toxicological model system. In the present study, we developed toxicity assays for mahi-mahi (*Coryphaena hippurus*), as an environmentally relevant pelagic marine fish species, and applied these assays to determine acute toxicity of perfluorooctanoic acid (PFOA), as a "legacy" PFAS, and perfluoro-3,6-dioxadecanoic acid (PFPrOPrA, or "GenX") as a next-generation PFAS, and possible environmental toxicant of concern. Bioassays demonstrated acute (24 h) toxicity, in the form of lethality, for both PFAS toward mahi-mahi embryos, and determined median lethal concentrations (LC_{50}) for both compounds. Probit analysis specifically determined mean 24-h LC_{50} values for PFOA and GenX to be 56 and 83 mg/L, respectively. Compared to zebrafish, mahi-mahi are significantly more sensitive to both PFAS, based on lethal concentrations (i.e., LC_{50} at 24 h of 56 versus 96 mg/L for PFOA, and 83 versus 126 mg/L for GenX, in mahi-mahi and zebrafish, respectively). This study showed that mahi-mahi represents a good environmentally relevant model for assessing environmental toxicants and reveals a higher sensitivity relative to zebrafish as an established laboratory model.

2169 Comparison of Routine Clinical Pathology Parameters of Cynomolgus Monkeys and Rhesus Macaque Monkeys

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Nonhuman primates are excellent models for studying biomedical research and routinely used in preclinical toxicity testing because of their genetic similarity to humans. Only a small number of species, such as macaques of the Cercopithecidae family of Old World monkeys, are well suited and established as translational models for drug testing. From this family, rhesus macaque monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) are the most common nonhuman primate animal models today. Although closely related, these two species show distinct phenotypic differences, morphology, behavior, and physiology. A comparison of common hematology and clinical pathology parameters between rhesus macaque monkeys and cynomolgus monkeys were performed. The results from the two data sets were analyzed using rhesus macaque monkey (*Macaca mulatta*) as reference control point. Analysis of the hematology data showed slightly higher white blood cell count (WBC), including absolute count of lymphocytes (LYMP), monocytes (MONO), and eosinophils (EOS, for males only) and slightly lower reticulocyte count (RET) in both sexes of cynomolgus monkeys when compared to rhesus macaque monkeys. Differences in clinical chemistry were restricted to relatively higher alanine aminotransferase (ALT) in cynomolgus monkeys relative to rhesus macaque monkeys. Whilst the data showed no major differences in parameters between cynomolgus monkeys and rhesus macaque monkeys it is strongly recommend that separate data sets are maintained for the interpretation of clinical pathology data.

2170 Continuous Tissue Glucose and Telemetric Cardiovascular Measurement in the Conscious, Freely Moving, and Socially Housed Cynomolgus Monkeys

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The Abbott FreeStyle Libre is the first for human use FDA approved (2017) continuous glucose monitor (CGM) that requires neither calibration with fingerstick measurements nor surgery. The device was used in combination with a small (13.7 g) cardiovascular telemetry devise (M11 PhysioTel®) for measurement of systemic blood pressure, body temperature and electrocardiogram. The goal of this study was to evaluate the feasibility of the device for the use in freely moving cynomolgus monkeys, and to evaluate the impact of restraint training on cardiovascular (heart rate) and glucose endpoints as potential stress biomarker. Four female cynomolgus monkeys (3.0 to 3.8 kg) minimal invasively implanted with the PhysioTel® M11 device for telemetric cardiovascular measurements were equipped with an external FreeStyle Libre CGM. Cardiovascular endpoints, body temperature and tissue glucose were measured in parallel while animals were repeatedly freely moving or physically restrained (infusion chair). Due to an unsuccessful tissue glucose measurement in all but one animal in the first instance, the experiment was repeated; thus phase I and phase II data were also compared. As expected, heart rate (HR) and tissue glucose increased during physical restraint. Mean HR were 130-150 bpm while animals were freely moving, and increased to up to 200 bpm during restraint, respectively. No HR acclimation was observed with repeated physical restraint. Mean tissue glucose increased transiently from 4 mmol/L when animals were freely moving to up to 8 mmol/L upon initiation of restraint. This pattern was repeatedly observed during independent phases I and II data collections suggesting full reproducibility. The Abbott FreeStyle Libre CGM is feasible for continuous monitoring of tissue glucose in freely moving cynomolgus monkeys for up to 8 days. Consistently increased cardiovascular and tissue glucose values as potential stress endpoints indicated a lack of any positive training effects. The study design did not clearly distinguish whether the increase in glucose levels was attributed to stress or more likely caused by increased muscle activity under restraint.

2171 Genetics of Global Göttingen Minipigs Colonies

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Selecting the correct nonrodent or large animal species for toxicology testing of pharmaceuticals in animals is very important to maximize human safety, clinical benefit, and animal welfare. Several factors should be considered in the large animal species selection process, including available scientific information and the need to balance scientific, ethical, and legal constraints. Göttingen Minipigs are a well-recognized large animal species balancing all needs for a translational animal model. This also includes a strict breeding management program ensuring genetic identity between the global Göttingen Minipigs breeding colonies in Europe, North America and Asia. The purpose of the presented study was to evaluate the efficacy of the breeding management program and to identify potential differentiation between the different breeding colonies due to genetic drift or unrecognized divergent selection of breeders in the five isolated breeding colonies. The study used whole genome re-sequencing of two representative DNA-pools per colony to access genomic variation within and differentiation between colonies. The data were completed with sequence data from 13 other pig breeds from public data repositories. An F-test was performed to reveal significantly differentiated allele frequencies between colonies; further, a pathway analysis was conducted. Variation within colony was quantified as expected heterozygosity. The results show that Göttingen Minipigs are easily discriminable from all other breeds, but with minor differentiation between the Göttingen Minipigs colonies. The differentiation results suggest that the underlying mechanisms for the minor differentiation are drift events rather than directed selection and limited to neutral genome regions. We conclude that the breeding management program maintains genetically stable and comparable colonies and shows no need for exchange of breeders between the colonies.

2172 Highly Sensitive Detection of Human Aspartate Aminotransferase Protein by AlphaLISA Is a Useful Way for Evaluating Hepatotoxicity in Humanized Liver Mice

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There are differences in drug metabolism, pharmacokinetics, and toxicity between humans and nonhuman species. To overcome these species differences, we developed a humanized liver (huLiver) mouse in which the hepatocytes of the host mouse liver were replaced with human hepatocytes. The huLiver mouse will be a valuable *in vivo* model for predicting hepatotoxicity in humans. However, since mouse hepatocytes remain in huLiver mouse, it is not possible to evaluate the degree of injury in human hepatocytes by alanine aminotransferase (ALT) activity because of the lack of species specificity. Therefore, we had been using human ALT, soluble keratin 18 protein, and circulating mRNA as human-specific biomarkers of liver injury. However, we could not observe the changes over time in these methods because of the requirement of a large amount of plasma (at least 50 µL/assay) caused by lower detection sensitivity. In this study, we aimed to establish a sensitive method applicable to huLiver mice. The AlphaLISA immunoassay kit for human aspartate aminotransaminase (AST) (PerkinElmer) was examined as a possible method to evaluate the hepatotoxicity in huLiver mice. We initially checked the species specificity of the human AST AlphaLISA detection kit using human and mouse samples. The AlphaLISA results for human AST showed slight cross-reactivity (<10%) against mouse plasma only in samples with high AST activity (>1,000 U/L). We administered a single intraperitoneal dose of thioacetamide (TAA, 200 mg/kg) to huLiver and control TK-NOG mice and analyzed routine parameters (ALT and AST activity) and AST protein levels in plasma at 0, 3, 7, and 24 hours. Overall, AST activities were correlated well with the activity of hepatic enzyme ALT in the corresponding samples (r^2 = 0.8088). In all cases, AST protein levels were higher in the huLiver mice groups than in the control TK-NOG mice groups. The kinetics of human AST protein was similar to AST activity in both huLiver (r^2 = 0.7464) and TK-NOG (r^2 = 0.7849) mice models. Furthermore, AlphaLISA could detect a significant increase of AST protein at 3 hours (5.5-fold) after TAA treatment in only the huLiver mice group with only 1 µL of plasma samples. Thus, human AST AlphaLISA can be a highly sensitive quantitative evaluation method for evaluating hepatotoxicity in a humanized liver mouse model.

2173 Comparative Toxicity of CD40 Antibodies in CD40/FcγR Humanized Mice and in Monkeys

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CD40 is constitutively expressed on antigen presenting cells and its activation leads to upregulation of costimulatory molecules, adhesion molecules and secretion of proinflammatory cytokines, and represents a promising approach to enhance anti-tumor responses. CD40 agonists have demonstrated clinical activity in patients with advanced solid malignancies, but treatment-related adverse effects, including thrombosis, cytokine release syndrome (CRS) and liver enzyme elevations were limiting escalation to potentially more efficacious doses. Identification and characterization of an improved and safer CD40 antibody relies on the availability of reliable non-clinical and models. Typical CD40 agonist antibodies bind to cynomolgus monkey, but not to mouse CD40 and some agonists also require cross-linking via Fc receptors for activity. Recently mice humanized for CD40 and Fc receptors were described and we used these mice to evaluate how they and cynomolgus monkeys informed risk assessment of CD40 agonists. For benchmarking purposes we generated a biosimilar version of a clinical stage agonist anti-CD40 antibody and tested this antibody in CD40/FcR humanized mice and in monkeys. When humanized CD40/FcgR mice were given a single iv dose of 0.5 mg/kg of this antibody (clinical MTD 0.2 mg/kg) decreased platelet counts and microthrombi in the liver and lung were detectable within 24 hours. CD40/FcR transgenic mice also provided mechanistic insight for the clinically observed hepatic effects and indicated ALT increases and hepatocellular damage were secondary to platelet activation, thrombus formation and infarcts. In contrast, when the same antibody was dosed to cynomolgus monkeys up to 5 mg/kg iv no ALT increases or thrombi were detectable. We obtained similar results with a number of BMS CD40 agonist antibodies; there were clear pharmacodynamic effects in monkeys, but no thrombosis or hepatotocicity. In contrast, CD40/FcR transgenic mice successfully identified safety concerns at relevant doses, including thrombosis and hepatotoxicity. These results support the value of using rationally selected humanized mouse models to enhance nonclinical safety assessment.

2174 Ovariectomy in an Animal Model Exposed to Environmental Toxicant Increases Susceptibility to Colon Polyp Development

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Colorectal cancer (CRC) is the third most common diagnosed cancer and the third leading cause of cancer-related deaths in the United States. The incidence of colorectal cancer and mortalities due to this disease are higher in men than in women. Epidemiological evidence revealed estrogens as protectors against the development of colon cancer in postmenopausal women treated with hormone replacement therapy. Previously, our lab has shown that male animal models develop twice as many polyps in the colon than female animals when exposed to the environment toxicant, Benzo(a)pyrene [B(a)P]. In addition, we have also shown that female animals exposed to B(a)P have an increased protein expression of the phase II drug metabolizing enzyme, Glutathione S-Transferase (GST) when compared to male animals suggesting the female sex hormones may regulate metabolism. The aim of this study was to determine whether ovariectomy in a female Polyposis in Rat Colon (PIRC) model increases B(a)P-induced colorectal tumorigenesis and whether tumorigenesis is reduced by estrogen supplementation after ovariectomy. Here, we show that ovariectomy in B(a)P treated (50 µg/kg bw) female PIRC rats increased colon polyp development compared to the vehicle. Our results also confirm that exogenous estradiol supplementation to ovariectomized female PIRC animals exposed to B(a)P significantly attenuates the development of colon polyps. This mechanism could involve the metabolism of the parent compound, B(a)P, by Glutathione S-Transferase and this pathway could be regulated by estradiol. *This research was funded by NIH grants 5R25GM059994-3, U54CA163069-04, G12MD007586-29, and 5RO1CA142845-04.*

2175 Aerosol Treatment for Ariway Hyperreactivity and Inflammation with an Inhibitor of Soluble Epoxide Hydrolase in a Murine Model of Asthma

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Asthma affects more than 300,000,000 people in the world today. An inhibitor of soluble epoxide hydrolase(sEH) is a potential method for the treatment of asthma. We studied the efficacy of an inhibitor of sEH, 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU). The goal of this study was to determine whether inhalation of TPPU could attenuate mucin secretion and airway hyper-responsivity in a murine model of asthma. To investigate an efficient method for the treatment of airway hyper-reactivity and inflammation, TPPU (1mg/ml) was administered by inhalation. Male BALB/c mice were given ovalbumin (OVA) by intraperitoneal (IP) injection on days 0 and 14 of the study as a means to sensitize them to allergen. Nebulized OVA (1%) was subsequently administered six times, every other day beginning on day 28 of the experiment, to provoke allergic inflammation and airway reactivity. Four treatments groups were tested: 1) PBS negative control, 2) OVA positive control, 3) OVA+TPPU (2h), 4) OVA+TPPU (6h). Pulmonary function was measured using a Flexivent system while applying increasing doses of methacholine, a potent bronchoconstrictor. At necropsy lung tissues were collected for quantitative measurements of lung inflammation, airway bronchoconstriction and the volume of intracellular airway mucosubstances. Semi-quantitative and morphometric measurements demonstrated following inhalation of TPPU for either 2 or 6 hours, a significant reduction in lung inflammation ($p<0.05$), intracellular mucosubstance volume ($p<0.05$) and airway contraction ($p<0.05$) in OVA-exposed mice. The average volume of intracellular mucosubstances per basal lamina surface area in OVA-treated mice was 0.0100±0.0007µm^3/µm^2. TPPU reduced mucosubstance volume by more than five-fold and four-fold following 2 or 6 hours of TTPU inhalation, respectively. The severity of airway constriction was three-fold lower in mice that inhaled TPPU for 2 or 6 hours, compared to the positive control. TPPU given by inhalation directly reduced airway hyper-reactivity, lung inflammation and mucin hypersecretion in this murine model of asthma. These findings suggest TPPU may be an effective treatment for asthmatic symptoms.

2176 Opioid-Induced Multi-Organ Necrosis in Ferrets

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Opioids are linked to both acute and chronic renal and hepatic damage following overdose exposures. Necrotic tissue lesions may be observed following opioid exposure in ferrets. A pharmacokinetics study was conducted in ferrets in which animals received multiple serial subcutaneous injections of the potent opioid carfentanil. Following each injection, study animals were monitored in whole body, unrestrained plethysmography chambers for 4 hours. During this time, the animals experienced multiple periods of apnea and respiratory depression, which causes hypoxemia and tissue hypoxia. Subsequent studies in ferrets monitored by telemetry have also demonstrated systemic hypotension associated with carfentanil exposure. Animals were kept on study for approximately 11 weeks and received a total of 3 or 4 injections of carfentanil. Gross findings at necropsy included multiple nodules on the surface of the kidneys, pancreas, small intestine, omentum, and peripancreatic fat. Microscopically, the nodules were composed of necrotic, saponified fat surrounded by a chronic-active inflammatory infiltrate. Nodules specifically overlying the pancreas were associated with areas of coagulative pancreatic necrosis. Saponified fat is commonly associated with pancreatic enzyme leakage following acute necrotizing pancreatitis. Additionally, diffuse acute and chronic renal tubular degeneration and necrosis with interstitial fibrosis were also observed, suggesting multiple cumulative incidents of acute kidney injury. Overall, multi-organ necrosis is hypothesized to be the result of tissue hypoxia caused by opioid-induced respiratory depression and systemic hypotension, and may be exacerbated in the kidney due to decreased renal blood flow.

2177 Aconitine Affects Behavioral Changes of Zebrafish Embryos via 5-HT Receptor

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Caowu (*aconite*) is a traditional Chinese medicine. Its main active ingredient is *aconitine*. Because it causes serious damage to the nervous system, it is regulated and used with caution. This experiment used zebrafish embryos as an animal model to investigate the neurotoxicity of aconitine to zebrafish embryos in early development. Experimental results showed that 100 μM aconitine can increase the number of coiling frequency in zebrafish embryos by 8-10 times, 5-HT1A receptor agonist 8-OH-DPAT also caused similar behavioral changes, however, when the 5-HT1A receptor selective antagonist WAY100635 was added, the increased coiling frequency behavior was inhibited. Exposure to aconitine also significantly increased serotonin receptor *5-htr1ab* and *5-htr1bd* mRNA expression. When the *5-htr1ab* mRNA is overexpressed alone, the zebrafish embryo also exhibits a significantly increased coiling frequency. Conversely, when the *5-htr1ab* gene is knocked down; the zebrafish embryo retrieval frequency is inhibited. Moreover, the protein expression of the serotonin receptor was reduced by a factor of 0.5 in the 96hpf (hour past fertilization) zebrafish embryo. The results suggest that the neurotoxicity caused by aconitine is mediated through the 5-HT receptor in the brain of the zebrafish embryos, which in turn changes the coiling behavior.

2178 A Nine-Month Inhalation Study to Investigate the Atherogenic Effect of Cigarette Smoke in Apolipoprotein E-Deficient Mice

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Atherosclerosis is one of the major causes of cardiovascular disease (CVD), including myocardial infarction, heart failure, and stroke. It has been reported that cigarette smoking is one of the risk factors for CVD and that smoking cessation results in a decline in the risk of CVD development. However, the atherogenic effect of cigarette smoke is not fully understood due to the lack of animal models available to investigate the effect of cigarette smoke on atherosclerosis development. To develop the model for the investigation on the atherogenic effect of mainstream cigarette smoke (MCS), female apolipoprotein E knockout (ApoE KO) mice were exposed to MCS for 9 months. In addition, mice were exposed to filtered air (FA) after MCS exposure to investigate the effect of smoking cessation on MCS-induced atherogenic changes. ApoE KO mice were exposed to FA or MCS generated from 1R6F Kentucky reference cigarette (600 μg total particulate matter/L) for up to 9 months using a whole body exposure system. In the cessation group, mice were exposed to MCS for 3 months before switching to FA for up to 6 months. ApoE KO mice were subjected to biological analysis at 3, 6, 8, or 9 months. Results showed that MCS exposure marker concentrations, such as COHb, nicotine and cotinine, in the blood of the MCS group were significantly increased, while those of the cessation group were similar to those of the FA group. No significant differences were seen in total plasma cholesterol and triglyceride concentrations between the groups at any time point. En face analysis showed that plaque surface areas in the aortic arch were significantly increased in the MCS group at 6, 8, and 9 months. In the cessation group, the aortic plaque surface area was significantly decreased compared with those in the MCS group at 8 months. In summary, this murine model could detect the acceleration of aortic atherosclerosis development caused by chronic MCS inhalation and smoking cessation retarded MCS-induced atherosclerotic progression in this murine model. These findings indicate that this model will be useful to investigate the effect of cigarette smoke on atherosclerosis development.

2179 Histopathological Characteristics in the PXB Hepatic Chimeric Mice: Comparison with SCID Mice

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PXB-mice are human hepatocyte chimeric mice originating from transgenic SCID mice generated by crossing urokinase-type plasminogen activator-transgenic mice with SCID mice. More than 70% of the liver of PXB-mice consists of human hepatocytes. When developing compounds for which clinical toxicity is difficult to predict by animal studies, PXB-mice may exhibit PK/TK profiling that is closer to that of humans than non-chimeric mice. Therefore, PXB-mice are expected to be used for toxicity studies in future. In order to conduct toxicity studies using PXB-mice, it is important to know the characteristics of this animal model. Histopathological examination was conducted in typical organs and tissues for toxicity studies in male PXB-mice (n=6, 23 to 24 weeks). The results were compared with those from male SCID mice (n=100, 12 to 20 weeks) that were the origin of the PXB strain. Many of the spontaneous histopathological findings in PXB-mice were also observed in SCID mice. Hypoplasia of the lymphoid organs was observed in both strains. A characteristic change in all PXB-mice was vacuolation in the hepatocytes, which was observed to various degrees. These were lipid vacuoles, and the vacuolated cells coincided with the human-derived cell region. There were no differences in the incidence and the degree of vacuolation among the hepatic lobes. In mouse-derived hepatocytes, no vacuolation was observed, although those were slightly larger than the average size of hepatocytes of SCID mice. In addition, the aorta of PXB-mice showed the focal intimal thickenings with an incidence of 50%. One PXB-mouse showed lymphoma in the various organs and tissues, and another PXB-mouse showed thymic lymphoma. PXB-mice, as well as SCID mice, were considered to be susceptible to thymic lymphoma. Based on the above results, it was suggested that PXB-mice are useful for an evaluation of toxicity, since there were no notable spontaneous lesions which hindered the assessment of hepatotoxicity risk in humans, except for 1 case of lymphoma.

2180 Comparison of Two Intravenous Infusion Methods in Freely Moving Beagle Dogs

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We compared two methods of ambulatory infusion in group-housed beagle dogs for feasibility and reproducibility. A catheter was surgically implanted in the femoral or jugular vein in four non-naïve female beagle dogs, and connected to an Instech® Orchestra ambulatory pump placed in the back pouch of a Ludomed® jacket worn by each dog. Sterile saline was continuously infused for 10 days (4.0 mL/hour). Saline bags were changed daily and the administered volumes were recorded. The dogs were group-housed and monitored daily for health status and to check integrity of the infusion system. Body weight and food consumption were recorded. The animals were euthanized on day 11, and a complete macroscopic examination was performed. Injection sites were examined microscopically at three sites: catheterized vein, tip of catheter and non-catheterized vein (1 cm from the catheter tip). At the femoral vein infusion site, both dogs showed iliac lymph node enlargement, with red discoloration or endothelial thickening. Minimal hemorrhaging was also noted in adjacent fat. These changes were considered to be non-adverse and consistent with commonly recorded findings at infusion sites. At the jugular vein infusion site, various microscopic findings were observed, including endocardial ulceration/hyperplasia, mononuclear/mixed inflammatory cell

infiltrates and myocardial/endocardial edema at the tip of the catheter and in the noncatheterized vein, both located in the right atrium. In addition, intimal thickening, endothelial ulcer and lymphoid cell/macrophage infiltrates in the vascular wall or thrombus and hemorrhaging in the endocardium were observed. In conclusion, implantation of an ambulatory catheter was well tolerated in the femoral vein, but implantation in the jugular vein caused local intolerance. Based on the findings from this study, it is considered that femoral vein catheterization provides a better method than use of the jugular vein and that this method is in line with the improvement of animal welfare during continuous infusion.

PS 2181 Clinical Pathology and Histopathology Parameters in Juvenile and Adult Guinea Pigs: A Comparative Study

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The guinea pig (GP) is a valuable animal model for nonclinical studies. Adult GPs express the human homologous *Kcnh$_2$* gene which encodes KCNH2 (or hERG1) protein, the major molecular target for QT interval regulation and drug-induced arrhythmias. The KCNH2 mediated cardiomyocyte repolarization is relatively unimportant in other traditionally used rodent animal models (rat and mouse), which renders the GP a valuable model for cardiovascular function evaluation in nonclinical studies. Additionally, for test articles that are pharmacologically inactive in other rodent models, the GP serves as an appropriate alternate. Also, the GP is one of the most suitable animal models for respiratory and otological nonclinical studies. One of the limitations of using GPs is the paucity of historical reference data. In this study, clinical pathology parameters from juvenile (8-10-week old; 22-155 males (M) and 22-148 females (F)) and adult GPs (30-week old; 5M and 5F), as well as histopathology evaluations from these two groups (juvenile: 5M and 5F; adult: 10M and 9F) were collected and compared. Compared to the juveniles, both male and female adult GPs have markedly higher total white blood cell (1.96-fold: M; 1.61-fold: F), lymphocyte (1.78-fold: M; 1.85-fold: F), monocyte (3.77-fold: M; 2.22-fold: F), basophil counts (7.73-fold: M; 6.09-fold: F), and fasting blood glucose level (2.08-fold: M; 1.86-fold: F), lower platelet count (0.57-fold: M; 0.62-fold: F), and shorter prothrombin time (0.74-fold: M; 0.76-fold: F) and activated partial prothrombin time (0.76-fold: M; 0.75-fold: F). Common histopathology findings in both juvenile and adult GPs include cardiomyocyte vacuolation, pigmented macrophages in cecum lamina propria, and inflammatory cell infiltration in multiple tissues. Of note, the prevalence of adipocyte infiltration in various tissues were observed only in adult GPs, i.e., brain ventricle (90%), salivary gland (89%), thymus (67%) and pancreas (67%), but not in juveniles. In summary, this study contributes to building a robust valuable historical control reference data set to aid in the planning for nonclinical studies. In addition, this study assures more accurate interpretation of findings when evaluating clinical pathologic and histopathologic parameters in juvenile and adult GPs.

PS 2182 Immunogenicity Study of HPV16L1/RG1 Chimeric Particles in Rabbits

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The primary objective of this pilot study was to determine and compare immunogenicity of HPV16L1/RG1 Chimeric Particle vaccines from two sources in rabbits (n=3/vaccine group). The vaccines were administered intramuscularly into the quadricep muscles of the hind legs on Days 1, 15, and 29. Blood samples collected from the rabbits on Days 15, 29 and 43 were analyzed for antibody production to HPV16L1 and HPV16L2 using the MSD platform. The titers of anti-HPV16L1 antibodies and *in vitro* neutralizing effect of these antibodies were greater with the vaccine produced by Paragon compared with the vaccine produced by Kirnbauer. The production of antibodies to HPV16L2 was lower compared to HPV16L1 in both vaccine products. Animals administered vaccines showed time-dependent increased levels of antibody production on Days 15, 29 and 43. The highest Ab titres were seen on Day 43. The vaccine produced by Paragon showed neutralizing activity for all three tested HPV subtypes (HPV16, HPV45, and HPV58) with the highest activity seen for HPV16. The neutralizing effect of the antibodies was confirmed in a mouse passive transfer assay. The rabbit antibodies fully protected (100% inhibition) all mice against HPV16, with the exception of one rabbit sera. Partial protection was observed for HPV45 and HPV58 subtypes; however, the viral load that the mice were challenged with was likely too large to allow for a thorough analysis of these results. In a separate study, different manufacturing processes of the vaccine where compared whereby one vaccine was subjected to the process of disassembly and reassembly (modified) to increase purity and the second vaccine was unmodified. The results indicated that the unmodified vaccine L1 antigen produced higher antibody levels on Day 43 after vaccination compared to the L1 in modified vaccine. L2 produced more comparable induction of antibodies following either of two vaccine immunization. In conclusion, both vaccines produced effective neutralizing antibodies for HPV16, as well as partial protection against HPV45 and HPV58. However, the Paragon vaccine appeared to be more effective in the production of antibodies (HPV16 L1/L2) compared to the Kirnbauer vaccine, based on the small number of animals in this study. In addition, the unmodified version had higher titers for L1 than the modified vaccine.

PS 2183 Differences in Survival, Incidence of Spontaneous Neoplasms, and Neoplastic Onset in Two-Year Carcinogenicity Studies Conducted in Crl:CD(SD) Rats and Crl:WI(Han) Rats

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Two commonly used rat strains in pharmaceutical development programs are the Charles River Crl:CD(SD) rat or CD®IGS rat, and the Crl:WI(Han) (Wistar Han IGS) or Wistar Han rat. To compare the performance of these two strains, the survival, incidence of major neoplasms that impact survival, and onset of these neoplasms in 2-year carcinogenicity studies were compared from data compiled from multiple Charles River Safety Assessment facilities from 2006 to 2018. These data show that the survival rate in CD®IGS rats has been declining from 2006 to the present. From 2006 to 2008, all carcinogenicity studies conducted in CD®IGS rats at Charles River facilities completed 95 weeks of dosing and 73% of these studies completed 104 weeks of dosing. However, from 2013 on, only 49% of carcinogenicity studies conducted in CD®IGS rats completed at least 95 weeks of dosing and just 15% of studies completed 104 weeks of dosing. In contrast, from 2006 to 2018, all carcinogenicity studies conducted in Wistar Han rats at Charles River Safety Assessment facilities completed the intended duration of 104 weeks of dosing. In fact, survival in Wistar Han rats at the end of Week 104 in 2-year studies is quite comparable to the survival of CD®IGS rats at the end of Week 80 in 2-year studies. Major neoplastic causes of death in CD®IGS rats include pituitary adenomas in males and females and mammary adenocarcinomas and fibroadenomas in females. In Wistar Han rats, the incidence of pituitary adenomas was 59% (females) to 65% (males) of that observed in CD®IGS rats, and the incidences of mammary adenocarcinomas and mammary fibroadenomas in females were 31% and 65%, (respectively) of those observed in CD®IGS rats. In addition, the onset of these neoplasms was two to four months later in Wistar Han rats compared to CD®IGS rats; as many of these neoplasms tend to result in early death late in the second year of a carcinogenicity study, this delayed onset confers a definite survival advantage to Wistar Han rats. These strain differences in survival and neoplastic incidence and onset strongly favor the use of Charles River Wistar Han rats in drug development programs in which eventual carcinogenicity testing in rats is anticipated (e.g. small molecule development programs).

PS 2184 A Simple Large-Animal Whole-Body Inhalation Exposure System to Study Environmentally Relevant Toxicant Exposures

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We developed a simple custom-made whole-body exposure chamber to study inhalation exposure to environmentally relevant concentrations of toxicants and/or infectious agents in a large animal model. Most whole-body inhalation exposure systems are designed for use in small animals such as rodents and rabbits. This is a limitation to research as exposures in non-rodent models is sometimes required, especially for translational studies. Minipigs have been recognized as potential animal models for human diseases. Currently, most large animal inhalation studies use anesthetized animals. However, anesthesia affects animal welfare, may confound research outcomes, and anesthetic gases are hazardous to investigators. To overcome these challenges, most inhalation studies in mini pigs utilize masks, which does not replicate real life exposure conditions. This is also challenging for prolonged exposures. In order to replicate real life conditions, we developed a whole-body inhalation chamber large enough to fit six 3-week-old Yorkshire Crossbred Isowean piglets. The 30 ft^3 transparent polystyrene exposure chamber is 76 cm W X 122 cm L X and 84 cm H. The chamber is fitted with valves to input breathing air or test gases/vapors, an exhaust valve to remove test and waste gases from the chamber. Animals have access to water and feed *ad libitum* and allows

for real-time measurement of chamber temperature, relative humidity, and oxygen. The clear chamber allows for behavioral evaluation during exposure. Using this chamber, we investigated the effects of low-level hydrogen sulfide (H_2S), an environmental stressor, on the pathogenicity of influenza A virus (IAV) in swine in a BSL2 facility. Thirty-five 3-week old pigs were exposed six hours daily for 12 days to either breathing air (BA) or H_2S in the chamber. On the seventh day, pigs were inoculated with placebo (NC) or IAV (C). Test groups were: BA/NC, BA/C, 0.5ppm/C, 5ppm/C, 50ppm/NC, and 50ppm/C. Results showed that pigs exposed to H_2S and inoculated with IAV had statistically significantly increased dyspnea, viral load, and microscopic lung injury compared to BA/C and reactive nitrogen species compared BA/NC group. However, there was a significant decrease in pro-inflammatory cytokines in lungs of pigs exposed to higher levels of H_2S. This novel custom-made whole-body exposure chamber can be adapted to study the effects of volatile toxicants, toxic gases, or infectious agents with appropriate containment in pigs.

PS 2185 Blood Sampling for Clinical Pathology in Rats: Retro-Orbital Sinus Sampling versus Jugular Vein Sampling

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Clinical pathology plays an important role in evaluating the potential toxicity of a test compound in a safety study. In these studies, at the end of the treatment with the test compound, blood is sampled from rodents (rats and mice) at to evaluate the effect of the test compound on haematology, coagulation and clinical chemistry parameters. Blood sampling via the retro-orbital sinus under isoflurane anesthesia was traditionally the method for clinical pathology blood sampling in rats. Recovery from the anesthesia can impact animal well-being and may impact the outcome of study endpoints. Blood sampling from the jugular vein without anesthesia has been considered an animal-friendly alternative method compared to retro-orbital sinus blood collection. As both methods have been used for collecting clinical blood samples, it is valuable to determine if differences in clinical pathology parameters are caused by the different blood collection methods in untreated animals. This study gives an overview of the normal ranges of clinical pathology parameters in untreated rats after blood sampling via the retro-orbital vein or jugular vein in different strains of rats (Wistar Han and Sprague Dawley). Differences in ranges between the two blood collection methods are discussed considering the differences in blood sampling location. This overview can be used to interpret differences in clinical pathology parameters in rat studies using these different blood collection methods.

PS 2186 Twenty-Eight-Day Continuous Intravenous Infusion in Mice: Use of Externalized Magnetic Ports and Tethers to Achieve Study Endpoints and Enhance Animal Welfare

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Continuous intravenous (IV) infusion in mice presents many challenges. The model requires intricate surgery and catheter size is limited due to vessel size. In addition, traditional tether security devices such as jackets, harnesses or tail-cuffs need to be continually checked and adjusted for proper fit. Improper fit of jackets or harnesses can lead to irritation, skin lesions, and swelling of the anterior body. Tail-cuffs can cause swelling and lesions where they are secured with steel suture. If the devices are too loose, the mouse can escape and destroy the exteriorized catheter, leading to patency issues and potential infection. A validation study was conducted comparing continuous infusion of 0.9% Sterile Saline for Injection (saline) and 20% hydroxypropyl-beta-cyclodextrin (HBC) in sterile water. Twenty-five CD1 male mice implanted with jugular catheters attached to an externalized port were purchased. Male mice were used since gender differences were not expected. The ports of 20 mice (10 per group) were connected to a magnetic tether/swivel assembly mounted to a counterbalance arm on the cage lid. The animals were infused at a rate of 2 mL/kg/hr for 27 or 28 consecutive days. Due to toxicity in 5 animals and a blocked catheter in 3 animals between days 5-10 in the 20% HBC group, the surviving and replacement animals were placed on a saline dosing holiday followed by dosing the remaining 10 or 17 days with 10% HBC. Animals were observed daily, and body weights, food weights and detailed physicals were collected weekly. Blood was collected for hematology and serum chemistry at the time of scheduled necropsy and histopathology was conducted on select tissues. All animals were patent for 27 or 28 days of dosing; 90% and 57% of the saline / HBC groups (respectively) were patent for blood draw at necropsy. There were no clinical, gross necropsy or histological signs of infection and white blood cell counts were normal. When both groups were compared to the historical reference, mean AST, ALP, SDH, and A/G ratio were slightly higher while mean globulin was slightly lower. The tether/port connection remained intact for all animals throughout the study and only one animal had a skin scab around the port beginning on day 14. The use of an externalized magnetic port and tether system proved successful in this 28-day continuous IV infusion study and was an improvement in animal welfare when compared to the use of jackets, harnesses, or tail-cuffs.

PS 2187 Adaptive Regulation of Bile Acid and Cholesterol Transporters in Mouse Kidney and Intestine during Alpha-Naphthylisothiocyanate (ANIT)-Induced Cholestasis

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Cholestasis is a decrease in bile flow that leads to accumulation in serum and liver of bile constituents such as bilirubin, bile acids, and cholesterol. It is known that cholestasis is associated with a decrease in biliary excretion of bile acids and alterations of bile acid metabolism and transport genes. We hypothesize that cholestasis results in an increase in non-biliary excretion of bile acids and cholesterol and alteration in the expression of bile acid and cholesterol metabolism related genes also occur in intestine and kidney. To test this hypothesis, eight-week-old male C57Bl/6 mice were administered alpha-naphthylisothiocyanate (ANIT) (75 mg/kg po) and liver, duodenum, jejunum, ileum, kidney, blood, urine, and fecal samples were collected 72 hours later. Serum levels of total bilirubin, total bile acid, total cholesterol, and ALP were elevated after ANIT. Hepatic bile acid and cholesterol concentrations were increased after ANIT. These results indicate that ANIT induces both cholestasis and hypercholesterolemia. Furthermore, fecal and urinary cholesterol and urinary bile acid concentrations were increased 72 hours after ANIT, whereas fecal bile acids were reduced. In liver, ANIT increased mRNA expression of sinusoidal efflux transporters Mrp3 and Mrp4 but decreased mRNA expression of Cyp7a1, Cyp27a1, Ntcp, Oatp1a1, Oatp1b2, and Bsep. In duodenum, ANIT increased mRNA expression of Mrp2 and the FXR target genes, Fgf15, Asbt, Ost-alpha, and Ost-beta. In jejunum, ANIT increased mRNA levels of FXR, Ost-beta, and the cholesterol uptake genes SR-B1 and LDL receptor, and decreased mRNA expression of Mrp3, Abca1, and Abcg5. In ileum, ANIT increased Mrp2 mRNA and decreased Mrp3 mRNA. In kidney, ANIT increased Mrp2, 3, and 4, Ost-alpha, and Ost-beta, and tended to increase SR-B1 and Abcg8. These changes in liver, kidney, and intestine may be an adaptive response to ANIT-induced cholestatic liver injury. Intestinal induction of LDL receptor and SR-B1, renal induction of Mrp2 and Mrp4, and increased tendency in renal SR-B1 and Abcg8 may promote fecal cholesterol, urinary bile acid, and urinary cholesterol excretion, respectively. These responses may facilitate alternative route for bile acid and cholesterol excretion during cholestasis.

PS 2188 Characterization of Glomerular Filtration Rate and Renal Blood Flow in Diabetic Miniature Swine

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People with type I diabetes (T1D) can have renal complications such as hyperfiltration and vascular restructuring that can potentially result in renal failure. The objective of this study was to determine if the induction of T1D in miniature swine impacted renal function by evaluating the glomerular filtration rate (GFR) and renal blood flow (RBF). Yucatan males (> 6 months old) were chemically induced into a diabetic state using alloxan. Three diabetic and three control animals were fasted overnight then administrated iodohippurate (15 mg/kg) and Iohexol (1.25 mg/kg), independently, via an intravenous injection. Blood samples were obtained prior to dosing and at 6 time points following dosing to evaluate the clearance rate of either iodohippurate (RBF) or iohexol (GFR). In the diabetic miniature swine, there was a 48% increase in GFR and a 43% increase in RBF, compared to control swine. The GFR increase suggests there may be a hyperfiltration phenotype in the alloxan-induced diabetic miniature swine, similar to what has been noted in patients with T1D. Furthermore, the increase in renal blood flow also suggests there may be some vascular restructuring occurring in the animal model. While further investigation into the correlation between renal dysfunction and diabetes will be needed, these preliminary data suggest the alloxan-induced diabetic miniature pig may be a good translational model for T1D renal function research.

2189 Combination of Lacto-*Bacillus* Formulation with Cyclosporine A Completely Prevents Disease Progression in a Murine Model of Inflammatory Bowel Disease

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Ulcerative colitis (UC) is characterized by chronic digestive track inflammation and immune system malfunction. We investigated the efficacy of Lacto*Bacillus* (3 strains combined) in an *in vivo* model of DSS colitis. Stool consistency, blood in the stool and body weight were evaluated and a total disease activity index (DAI) was calculated. Colons were collected for cytokine and histopathology evaluation. Bacterial dosing was performed daily at $1.5x10^{10}$ cfu/mL alone or in combination with Cyclosporin A (CsA). Fecal samples for microbiome analysis were collected. Treatment with both CsA and live bacteria significantly lowered DAI score when compared to control, consistent with improvements in the colon length and composite histopathology score. The combination of live bacteria + CsA had superior effect on in-life scoring and histopathology findings. Majority of animals assigned to this group had no signs of inflammation or disease in colon. Decreases in anti-inflammatory cytokines including IL-2, Il-6, Il-17, KC/GRO and TNF were observed in colon of animals receiving live bacteria formulation or CsA alone. Consistent with in-life scoring and histopathology, additive effects were observed for combined therapy (live bacteria + CsA). Changes in fecal microbiome have been observed during the course of the study. There is an apparent slight improvement, where an apparent shift towards baseline microbiome composition was observed in treated animals compared to DSS control. Our data clearly indicate treatment of mice with live therapeutics by oral gavage significantly prevents development of DSS-induced colitis in mice with no significant safety concern. Combination of live therapeutics with a standard of care compound (CsA) had an additive effect with almost a complete prevention of the disease. These bacterial strains alone or combined with SOC present an alternative approach in treatment of colitis in clinic.

2190 Cerebrospinal Administration in the Juvenile CD1 Mouse Using the Intracerebroventricular Route

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An increasing number of molecules targeted to address neurological diseases and deficiencies are in development. As these are often larger molecules which fail to reach their target due to low penetration of the blood-brain barrier and/or have short half-life in circulation, targeted administration of these drugs directly into the central nervous system is often indicated. Neurological disorders often appear or develop during childhood and there is a heightened interest in conducting nonclinical studies in juvenile animals to mimic the age and development stage of the target population. Administration into the intrathecal space is more difficult than routine approaches regardless of species, but these challenges are greater when attempting these in smaller species such as the mouse, and more so in a juvenile population. In order to circumvent the technical limitations for intrathecal administration due to the size and delicate anatomy of the juvenile mouse, our laboratory developed an approach to conduct repeated intracerebroventricular (ICV) injection in CD1 mice of 21 days old. Anesthetized mice were restrained, and dosing was guided using stereotaxic table and predetermined coordinates to target the right ventricular area. During trials, a portion of mice were used for confirmation of successful injection. Diluted ink or saline was injected in the ventricle, and accuracy was evaluated visually following euthanasia and extraction of the brain from the skull and performing a coronal cut. Following this confirmation, an additional subset of mice was injected again 7 days later to assess tolerability of repeated anesthesia and injection. These animals were then maintained for an additional week. This subset was then euthanized, the brain was collected and histopathological evaluation was performed in order to characterize accuracy of the injection, and any procedure-related findings. There were no abnormal clinical signs and no changes in body weights or food consumption noted following the first or second injection. Animals recovered well from the procedures, and histopathology evaluation showed only minimal background findings. These results demonstrate that the ICV route of administration can be successfully used for safety assessment studies using cerebrospinal delivery in the juvenile mouse.

2191 Impact of Anesthesia on Rat Clinical Pathology

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Clinical pathology is a critical endpoint for preclinical systemic toxicity studies. In 2017, AAALAC recommended reducing the flow rate of CO_2 to a 10-30% displacement rate when anesthetizing rats. This may impact clinical pathology values. The objective of this study was to evaluate clinical pathology parameters using different anesthetics prior to terminal blood collection in rats. Thirty Sprague Dawley® rats were randomized to three groups. Ten animals (5 M/ 5 F) were evaluated per group. Rats were fasted and placed in an induction chamber using: 1) 4% isoflurane in O_2, 2) CO_2 with a 30% cage volume displacement per minute (30% CO_2) or 3) CO_2 with a 70% cage volume displacement per minute (70% CO_2). Following anesthesia, blood was collected by cardiac puncture into EDTA and SST™ Vacutainer® tubes (BD, Franklin Lakes, NJ). Whole blood was assayed for hematology parameters using the Hemavet 950 (Drew Scientific Inc, Miami Lakes, FL). Serum was assayed for clinical chemistry parameters using the Vet Axcel® (Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell, NJ). Results were compared among groups using multiple unpaired t-test. Probability (p) values < 0.05 were considered statistically significant and biological relevance was evaluated. Statistically significant differences were observed among groups for the time to accomplish anesthesia. Exposure time in minutes was shortest for 70% CO_2 (4.2 ± 0.4), followed by 4% isoflurane (5.2 ± 0.8) and finally 30% CO_2 (6.0 ± 0.5). Compared to isoflurane, 30% CO_2 had a statistically significant impact on at least 11 clinical chemistry parameters, with glucose, phosphorus, potassium (all increased) and chloride (decreased) having biologic relevance. These parameters were also impacted when 70% CO_2 was used, but to a lesser magnitude. Prolonged exposure time at the lower CO_2 flow rate created a respiratory acidosis leading to electrolyte imbalance. Anesthesia with 30% CO_2 had no significant impact on hematology parameters. A trend of decreased leukocyte count was observed. A larger group size is needed to fully determine biologic relevance. Hematology parameters were similar at 30% and 70% CO_2. Choice of anesthesia must be considered when comparing clinical pathology data among laboratories. Artifact produced by preanalytical variation must be considered. Since clinical chemistry parameters collected under isoflurane were closer to published ranges, and CO_2 anesthesia resulted in some values incompatible with life, isoflurane is recommended when evaluating clinical pathology endpoints.

2192 A Mouse Model of *In Utero* Ultrafine Particulate Matter Exposure and Infant Respiratory Syncytial Virus Disease

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In utero exposure to particulate matter (PM) air pollution has been associated with increased lower respiratory tract infections (LRTIs) in infants. Despite the known sensitivity of the fetus to environmental pollutants and epidemiological evidence correlating prenatal PM exposure and LRTI morbidity, mechanisms of PM enhanced pathogenesis are relatively unexplored in immunologically immature populations. Moreover, the role of ultrafine particles (UFP, ≤ 0.1 μm) in the etiology of childhood respiratory disease are limited. To clarify the impact of *in utero* UFP exposure on LRTI morbidity, we exposed time-mated C57BL6/N mice to 100 μg/m^3 ultrafine particulate matter (LD), 500 μg/m^3 ultrafine particulate matter (HD) or filtered air (FA) for 6-h daily from gestational day 0-18. At 5 days of age, offspring were challenged with culture media (sham control) or a chimeric strain, rA2-19F, of respiratory syncytial virus (RSV) previously shown to elicit an aberrant immune response similar to infant infection. At 3 and 11 days of age, offspring were examined for pulmonary inflammation, via histologic and bronchoalveolar lavage (BAL) fluid analysis, pulmonary T cell profiles, and viral load (3 dpi). Blinded histologic examination revealed an increased inflammatory response within the RSV dosed offspring, with a subjectively heightened inflammatory response of the HD and LD exposed mice in comparison to the FA exposed mice. Within RSV dosed offspring, LD exposed offspring had significantly increased total leukocytes within BAL fluid compared to FA exposed mice. Eosinophilia varied within and between all exposure and dosing groups, with no significant trends. Viral load and flow cytometry analysis is ongoing. In conclusion, it appears the PM exposed, RSV dosed offspring exhibit an increased inflammatory response.

2193 The Value of History of Use within the Safety Assessment of New Dietary Ingredient Notifications (NDins)

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The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires that manufacturers and distributors of a dietary supplement containing a new dietary ingredient (NDI), that has not been present in the food supply as an article used for food, submit a premarket safety notification to the FDA at least 75 days prior to introducing the ingredient to the market. The codified rules for the data required to be in a New Dietary Ingredient Notification (NDIN) (21 CFR 190.6) include a "history of use or other evidence of safety establishing that the dietary ingredient, […] will reasonably be expected to be safe…" The safety assessment within an NDIN review can be viewed as a modified hazard assessment where the history of use (HOU) serves as the exposure assessment and also provides insight into hazard identification. Specifically, a well-documented history of safe use serves several functions within the NDIN safety assessment: 1) provides documentation of historical consumption for comparison between the proposed conditions of use of the notified ingredient or supplement product to documented historical values; 2) helps to identify the data gaps which can be filled with other evidence of safety or safety studies; and 3) can be used to modify the safety factors used in safety calculations. New dietary ingredients vary from those with well-documented histories extending hundreds of years to those which have no history of use. Therefore, the data submitted for these ingredients will vary significantly depending on the HOU data available. A majority of the unique NDINs' received since 2014 contain HOU data in addition to other evidence of safety, but the HOU does not have the same substantive value in all NDINs. Among NDINs which were acknowledged without objection since 2014, approximately 38% contained a HOU which contributed to the safety review conclusion by modifying safety factors from pre-clinical animal data or establishing a reasonable expectation of safety based on historical consumption. Case studies of how history of use data affected the safety assessment and ultimately the conclusion of the NDIN review will be presented. While often it is a balance of both history of use data and other evidence of safety data that results in a reliable basis for an expectation of safety, the history of use, whether representing years of marketing success or centuries of experience, plays a necessary and significant role in the conclusion of the NDIN safety assessment.

2194 Screening Hazard Information for Low-Priority Substance Chemicals under the Toxic Substances Control Act (TSCA)

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Under the Frank R. Lautenberg Chemical Safety for the 21st Century Act amendments to the Toxic Substances Control Act (TSCA), the US Environmental Protection Agency (EPA) is directed to prioritize existing chemicals in the TSCA Inventory as either high-priority substances for risk evaluation or low-priority substances for which risk evaluations are not warranted at the current time. The statute required EPA to designate 20 low-priority substances and 20 high-priority substances by December 2019. EPA created a fit-for-purpose literature search and review approach to transparently identify and document relevant and reasonably available information to support the risk-based screening review of the low-priority substances. Here, we outline EPA's approach for identifying, screening, evaluating, and integrating hazard (human health and environmental) and fate information to support the first 20 TSCA low-priority substance designations. Specifically, we describe sources, tools, inclusion/exclusion criteria, and data quality metrics used to identify relevant references for the screening reviews. For each chemical, EPA searched for hazard information using five search engines to access peer-reviewed literature (e.g. PubMed, Web of Science) and more than 20 "grey" literature and additional sources (e.g. online data repositories). All references were managed using the Health & Environmental Research Online (HERO) database and DistillerSR software during title/abstract screening, full text screening, and data evaluation steps. If data for human health or environmental endpoints were of insufficient quality or were unavailable for the target chemical, modeling data were used to fill data gaps or analogs were identified for read-across. Analog references were evaluated using a selective application of the same screening and evaluation strategy used for grey literature and additional sources. From the title/abstract screening, full text screening, and data evaluation steps, on average 3%, 43%, and 60% of references were included. Of the acceptable sources used for final designation, approximately 79% were from grey literature sources and 21% were from primary data sources. The approach described above was used to support the first 20 low-priority substance designations under TSCA and provides insight on the data that supports assessment of low-hazard chemicals.

2195 Nonclinical Regulatory Guidelines Development: EU, USA, and Australia—Regulatory Point of View

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Preclinical studies, also known as the preclinical development and nonclinical studies, is a phase of research that begins before clinical trials, and during which important feasibility, iterative testing and product safety data are collected. The aim of this study is to highlight the most important well-established regulatory nonclinical guidelines and create the way for other researchers across the globe to investigate these guidelines and regulations. Also, promote harmonization of the preclinical studies to support human clinical trials and reduce differences between regions. A review was done on the updated guidelines of the following: European Medicines Agency (EMA), United States Food and Drug Administration (US FDA), and Therapeutic Goods Administration (TGA) of Australia. The content was reviewed and analyzed**.** Based on the collected information from multiple regulatory authorities with a wide range of stringency in terms of preclinical regulatory guidelines, it was noticed that most of the regulations referred to the International Council for Harmonization (ICH) guidelines. In summary, the findings indicate the need for more harmonization to standardize the guidelines across the globe. In addition, studies and collaborations are necessary in the area of nonclinical to reduce the requirements, efforts and therefore maintain the resources and unify the regulatory themes.

2196 Toxicology Studies to Support a Clinical Trial Using [^{68}Ga]-Labeled Radiotracers for PET Imaging

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During last years, the Positron Emission Tomography (PET) tracer technology is becoming more of interest to be used in clinical and preclinical investigational studies. Some typical questions to be answered with this technology are imaging of tissues (e.g. tumors) specifically targeted with the radiotracer, bio-distribution of new radiolabel drugs or occupancy assessment of drug target receptors. In our case, two new [^{68}Ga] gallium-labeled PET radiotracer compounds (binding and active agonists at GLP-1 or Glucagon receptor) were intended to be used in a clinical study to investigate the receptor occupancy of a dual GLP1/GCG receptor agonist. Structurally, the radiotracers consist of a peptide strain which bound to a DOTA chelator which acts as the complex binding side for the [^{68}Ga] gallium isotope. The dual agonist will be given during a 4-week dosing period by daily SC administration and each of both PET radiotracers will be administered to the human subjects as short IV infusion once at base line and a second time at the end of the 4-week dosing period. PET scans will be performed after radiotracer administrations. A meeting with Swedish Medical Product Agency (MPA) took place in advance of the non-clinical testing to discuss the appropriate toxicology study design. Sanofi proposed both compounds would fall under the micro-dosing approach according to ICH M3 (R2) guideline and discussed specifically the micro-dosing requirements to be fulfilled for clinical radiotracer testing: total dose given ≤ 100 µg; total dose ≤1/100th of NOAEL (in mg/kg); total dose ≤1/100th of pharmacologically active dose (scaled in mg/kg). Further, Sanofi proposed to use unloaded tracers (neither loaded with labeled [^{68}Ga]$^{+3}$ or Ga^{+3}) to be tested in each of the extended single IV dose toxicity rat studies in the rat and use dose levels of the unloaded tracer which correlated to a 10-, 100-, or 1000-fold of the intended clinical dose of radiotracer. Agency agreed to the use of unloaded tracers molecules for animal testing; also to the proposed toxicology study design and to the strategy that radiotracers - in our clinical study setting - would fall under micro-dosing approach number 1 of ICH M3 (R2) guideline requiring extended single dose toxicity study.

2197 First Results of Developmental Neurotoxicity (DNT) Testing in the Extended One-Generation Reproductive Toxicity Study (EOGRTS) under the REACH Regulation

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REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) is a regulation of the European Union, to protect human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. Under REACH, the EOGRTS

(OECD TG 443) is an information requirement for higher tonnage substances. The EOGRTS design is concern-driven and specified to meet risk assessment and classification and labelling needs. The DNT cohorts 2A and 2B of EOGRTS are required if the available information on a substance indicates a particular concern on (D)NT. Evidence supporting these concerns could originate from existing information derived from *in vivo* or non-animal approaches, from the knowledge of relevant mechanisms or modes of action of the substance itself, or from information on structurally analogous substances. Since the implementation of EOGRTS, the first results from requested studies with DNT cohorts have become available for the following substances: (A) diethanolamine, (B) 2-ethylhexanoic acid, (C) the oligomerisation and alkylation reaction products of 2-phenylpropene and phenol, (D) 2-(4-tert-butylbenzyl) propionaldehyde, (E) 4,4′-propane-2,2-diyldiphenol, polymer with 2-methyloxirane, (F) biphenyl, and (G) 3,5-dimethylpyrazole. Effects were observed for substances A, C, D and F. For substance A, adverse clinical observations, impaired auditory startle response and corresponding neuropathological findings were observed. For substance C, morphometric evaluation of the brains of Cohort 2A rats revealed lower hippocampus thickness values in high-dose males, and lower Cornu Ammonis thickness values in high-dose females (both when measured unilaterally). For substance D there were some small effects on brain weight, and altered auditory startle response. For substance F some effects on grip performance were reported for high-dose animals. These are the first results of DNT-triggered testing under REACH based on a limited number of studies for which results have become available on ECHA's dissemination website. Additional data is generated because DNT cohorts are requested in approximately 25% of ECHA decisions.

2198 Developing a Defined Approach for Eye Irritation Testing

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Regulatory acceptance and implementation of new approach methodologies depend on public-private partnerships, which allow communication and cooperation among federal agencies and the private sector. To that end, the PETA International Science Consortium Ltd., the Interagency Coordinating Committee on the Validation of Alternative Methods, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, and CropLife America companies are collaborating on a three-phase evaluation to assess the applicability of *in vitro* eye irritation test methods to assess eye irritation potential for agrochemical formulations. Six formulations with existing *in vivo* data, classified as non-irritating (US Environmental Protection Agency [US EPA] Category IV) or severely irritating (US EPA Category I) were tested in Phase 1. The formulations were tested in the bovine corneal opacity and permeability (including histopathology), neutral red release, isolated chicken eye (including histopathology), EpiOcular (eye irritation test and time-to-toxicity protocols), and porcine cornea reversibility test methods. Each method predicted the same category as the rabbit test for most of the tested formulations, showing promise for further testing. Ten additional agrochemical formulations with *in vivo* data, representing a wider range of eye irritation classifications (US EPA Categories I, II, III, and IV), were evaluated in Phase 2. While none of the methods directly correlated with the *in vivo* results, several methods showed potential for use in a defined approach to assess agrochemical formulations. Phase 1 and 2 results will be used to identify which methods will be evaluated in Phase 3 and can form the basis of a defined approach for testing of agrochemical formulations for eye irritation potential. *This project was funded with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.*

2199 Integrated Regulatory Strategy to Prioritize Per- and Polyfluoroalkyl Substances (PFAS) of High Concern and Define Risk Management

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The European Chemicals Agency (ECHA) Integrated Regulatory Strategy aims to ensure the coherent implementation of the regulatory processes and supports authorities in addressing substances of concern. Coherent regulatory processes also contribute to meeting the 2020 goals of the World Summit on Sustainable Development. Therefore, with the support from Members States, ECHA has moved from a substance-by-substance assessment to assessment of groups of structurally and/or functionally similar substances. Per- and polyfluoroalkyl substances (PFAS) constitute a large group of substances widely used in various consumer and industrial applications. A few PFAS and salts of PFAS have recently been the subject of regulatory action in Europe, including harmonised classification as developmental toxicants, identification as substances of very high concern (SVHC) and restriction. In order to clarify common structural and developmental toxicity properties and to propose regulatory strategies for groups of PFAS, we mapped these chemicals within the chemical universe of REACH registered substances (chemical universe) and also substances notified in the C&L inventory. Algorithms were developed using a set of specific chemical features. Any notified or registered substance for which ECHA has at least one dossier with a composition that contains a constituent, impurity, or additive with a molecular structure that contains at least one C-F bond was located. Currently, the focus is on PFAS substances mapped in about 30 structurally-related subgroups. The analysis shows that the complex toxicity profile of PFAS is better understood at a subgroup level, provided that some data are available. The analysis attempts to determine the presence of structural alerts in some PFAS and to apply read-across for some subgroups, to investigate whether exposure to structurally similar PFAS results in similar health effects in order to facilitate an integrated regulatory strategy. These finding may also support informed substitution. Additional studies on the critical effects will facilitate read-across and may avoid regrettable substitutions.

2200 Rethinking Carcinogenicity Assessment for Agrochemicals

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For the past 40 years, questions have been raised about the relevance and regulatory utility of rodent cancer bioassays in human health risk assessment. As a result, a working group of experts from different sectors have formed the Rethinking Carcinogenicity Assessment for Agrochemicals Project (RECAAP) to determine the appropriateness of and criteria for waiving rodent cancer bioassays for the registration of food-use pesticides. A weight of evidence (WoE) reporting framework, which outlines a suggested assessment of publicly available information, was used to draft carcinogenicity study waivers to determine if sufficient information was available to perform a health protective chronic risk assessment without conducting rodent cancer bioassays. Information used in the WoE include exposure, mode-of-action, physiochemical properties, metabolism, and sub-chronic toxicological data from standard risk assessment endpoints. Using this framework, RECAAP evaluated 29 pesticides registered over the past ten years with the chemical distribution spanning 15 tumor types, 28 chemical classes, and eight cancer classifications (including subclasses). The reporting framework criteria and example carcinogenicity waivers will be presented. This effort has established criteria for when the mouse and/or rat cancer bioassay can be waived while ensuring that pesticide human health risk assessments are protective.

2201 Assessing the Impact of Expert Knowledge on ICH M7 (Q)SAR Predictions. Is Expert Review Still Needed?

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The ICH M7(R1) guideline recommends the use of complementary (Q)SAR models to assess the mutagenic potential of drug impurities as a state-of-the-art, high-throughput alternative to empirical testing. Additionally, it includes a provision for the application of expert knowledge to increase prediction confidence and resolve conflicting calls. Expert knowledge, which includes structural analog searching and mechanistic interpretation, has been particularly valuable in situations where models return an indeterminate (equivocal) result or are unable to generate a prediction due to a lack of relevant training set analogs (out-of-domain). Recently, a retrospective analysis was conducted by FDA's Center for Drug Evaluation and Research to assess the impact of

applying expert review to (Q)SAR predictions for 1002 drug impurities evaluated in new and generic drug applications between April 2017 and April 2019. Expert knowledge overturned the default predictions for 26% of the impurities and resolved 96% of equivocal predictions and 75% of out-of-domain calls. Of the 261 cases where expert knowledge overturned the default prediction, 15% were upgraded to equivocal or positive and 79% were downgraded to equivocal or negative. Furthermore, the chemical classes where this effect was most pronounced were polycyclic aromatic systems (68% overturned), aldehydes (39% overturned), aromatic amines (35% overturned), and Michael-reactive acceptors (33% overturned). Collectively, the results suggest that a modified workflow that triages predictions could be used to improve (Q)SAR review efficiency, and that the application of expert knowledge still plays an important role in an ICH M7 (Q)SAR prediction workflow.

PS 2202 Phage Therapy as a Viable Treatment Option to Overcome Drug Resistance: Nonclinical Regulatory Progress and Scientific Perspectives

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Superbugs that are immune to multiple drugs are a concern with more frequent use of antibiotics. Phage therapy is considered as an alternate to overcome bacterial multiple-drug resistance (MDR), yet there is no guidance in making this a reality. Aim of this presentation is to review the evolution of phage therapy and to share the lessons learned (educational) by the authors' "nonclinical regulatory" experience. To this end, pertinent literature was reviewed, expert opinions were pursued, and authors' personal experiences discussed. Results showed: phage therapy is enlisted as biologic, but there are differences from "biologics", i.e., phage is a living and replicative virus, generally non-toxic/well tolerated, which targets directly and specifically bacterial envelope, uses the host bacteria to multiply, is self-dosing (multiplication linked to bacterial population dynamics) and does not trigger a significant immune response to itself (as opposed to live viral vaccines). However, there is a toxicological concern such as risk of toxic shock as result of dead bacterial debris and release of toxins. Proof of efficacy in *in vitro* and pharmacology models, limitations of animal toxicology studies considering the 3R-principles have strengthened the relevance of alternative models as pivotal for pharm/tox regulatory pathway to a marketed drug. With technological advancements, particularly in Good Manufacturing Practices, there has been an increasing trend in clinical studies of patients with severe bacterial infections, successfully treated with phages as compassionate-use, providing last hope for patients suffering from MDR infections. Increased rate of clinical success raised awareness in the stakeholders, so workshops were held by both EMA (2015) and FDA (2017) to exchange information with the medical and scientific community about the issues associated with bacteriophage therapy. Recent reviews on this topic highlight a strategic collaborative effort globally to improve this with a rationalized scientific approach. Despite the rapid increment in finding relevant *in vitro* and *in vivo* pharmacological models, alternative toxicity testing with clinical relevance and continuously expanding clinical applications, a specific regulatory guidance on this topic is needed. As such, the interaction with regulatory bodies is currently a "negotiation" process, using scientific arguments. Based on authors' experience (up to 5), it is concluded that such interactions have helped/will help charter a pathway to bring phage therapy as a valuable treatment option to the patient.

PS 2203 A Retrospective Study on EU Harmonized Classifications for Carcinogenicity to Guide Future Research

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Under European Regulation (EC) No 1272/2008 (CLP), chemicals can be defined as carcinogenic if they induce tumors, increase tumor incidence and/or malignancy, or reduce the time to tumor development. In January 2019, 149 reports considering the carcinogenicity classification of chemicals under CLP were identified on the ECHA website, covering two Carc. 1A chemicals ("known to have carcinogenic potential for humans"), 24 Carc. 1B ("presumed to have carcinogenic potential for humans"), 44 Carc. 2 ("suspected human carcinogens") and 79 chemicals not classified for carcinogenicity. The general features of these ECHA opinions were considered. Human carcinogenicity data resulted in two chemicals being classified as Carc. 1A, and supported the classification of 29% of Carc. 1B substances. Most (71%) Carc. 1B chemicals induced tumors in more than one species (mainly rodent, but also human, data). For 29%, tumors in a single rodent species were sufficient for 1B classification in WoE assessments (e.g. also considering genotoxic potential). For Carc. 2 classifications, 59 and 41% were based on tumors in a single species or in two rodent species, respectively. Of the chemicals not classified for carcinogenicity, 11% had no relevant data available, while others were not classified based on substance-specific or read-across data indicating a lack of effect (66%). A further 23% had indications of carcinogenicity in rats and/or mice which were not considered relevant to humans. Carc. 1B chemicals most frequently induced tumors in the rat lung (33%) or nasal cavity (25%), and/or the mouse lung (38%) or liver (33%). For Carc. 2 chemicals, the most frequently affected organ was the liver (32% of Carc. 2 chemicals in rats, 30% in mice), and Leydig cell tumors were also very commonly induced in rat testes (27%). In mice, besides the liver, the most common target was the lung (16%). Twelve of the chemicals not classified for carcinogenicity produced tumors in rats; four affected the liver, four the thyroid, two the testes and two the mammary gland, while the preputial gland, blood and adrenals were affected by one chemical each. In mice, six 'not classified for carcinogenicity' chemicals induced tumors in the liver, while the thyroid, lung and Harderian gland were affected by one chemical each. Based on summarized MoAs linked to cancer classification, an assessment of the different tumor sites affected by the 44 Carc. 2 chemicals was conducted to present prioritized areas for future MoA-based research.

PS 2204 Assessment Strategy for the Identification of Endocrine Disruptors

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For the first time, ECHA and EFSA have jointly published a Guidance document [1] on how to identify endocrine disruptors in accordance with the scientific criteria for the determination of endocrine disrupting (ED) properties applicable to the Biocidal Products (BPR) and the Plant Protection Products (PPPR) Regulations. The ED criteria are unique in that they are the first translation of the WHO/iPSC definition of an endocrine disruptor into regulatory requirements. According to the criteria a substance shall be considered as having ED properties: if it shows an adverse effect, if it shows endocrine activity, and if there is a biologically plausible link between the adverse effect and the endocrine activity (i.e. it has an endocrine mode of action). They require a weight of evidence approach for reaching the conclusion that includes taking into account all available information and conducting a mode of action analysis (which includes assessing essentiality, consistency and specificity). Separate conclusions are required on whether the ED criteria are met with respect to humans and non-target organisms. Depending on the available information, additional data might need to be generated on order to reach these conclusions. The data requirements of the BPR and PPPR contain more mammalian studies that may be informative on ED properties than studies on other taxonomic groups. Thus, in line with the general principle to avoid unnecessary animal testing, the assessment strategy in the guidance recommends to strive for a conclusion on the ED properties with regard to humans first, followed by a conclusion on mammals as non-target organisms based on the same data set. Only when the ED criteria are not met for mammals as non-target organisms, there will be a need to proceed to other taxonomic groups. First experiences with the practical application of the ED Guidance are discussed including the outcome of the first ED conclusions on active substances. *Reference: [1] Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 https://doi.org/10.2903/j.efsa.2018.5311.*

PS 2205 Current and Future Risk Assessment Considerations for Genotoxic and Nongenotoxic Carcinogens

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The National Academies of Science risk assessment paradigm includes both qualitative hazard identification and quantitative dose-response assessment, which are combined with exposure assessments to render a risk characterization to inform risk decisions. An often-mischaracterized aspect of cancer dose-response assessment is in the latitude for risk model selection between genotoxic versus non-genotoxic carcinogens. The 2005 EPA Cancer Guidelines recommend a health-protective default assumption of a linear low-dose model where any exposure poses some risk; the question is then what level of risk is acceptable. Typically, risks lower than one-in-a-million ($1:10^6$) are deemed to be acceptable; however, higher risk levels may sometimes be deemed acceptable considering technical feasibility to reduce exposures, and social, cultural and economic factors. The most often proposed alternative "non-linear" risk estimation models assume a threshold exposure level to be achieved and maintained in order to induce cancer. In a thresh-

old model, any exposures below a certain level are assumed to not increase cancer risk, and estimations of risk are then similar to estimates for non-cancer risks. A common misconception is that the Cancer Guidelines (EPA, 2005) assume a linear low-dose model only if a carcinogen is active through a genotoxic mechanism, and a non-linear (e.g., threshold) model if it causes cancer through a non-genotoxic mechanism. In actuality, critical mechanistic evidence is needed before replacing the default assumption of linearity with any other model to estimate risks, regardless of whether a genotoxic or non-genotoxic mechanism is the putative cause. Several examples in completed cancer risk assessments will be presented to illustrate aspects of these considerations. As greater understanding is gained on the specific mechanisms leading to cancer and those individual mechanistic steps are combined to create credible adverse outcome pathways (AOPs), it is anticipated that the evidence to either support or reject the default linear assumption will be enhanced. These considerations also need to be made in the context of exposures of populations to many agents contributing to overall cancer risk, including occupational exposures as well as exposures in ambient air, drinking water and food.

2206 Comparison of Flavor Chemicals and Cytotoxicity of Authentic and Counterfeit Refill Fluids Purchased Worldwide

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Electronic cigarettes (ECs) expose consumers to nicotine, flavor chemicals, metals and reaction products that can negatively impact the health of users. Recently, the use of ECs has been linked to vaping-associated pulmonary illness (VAPI). To examine the relationship between flavor chemicals and VAPI, we identified and quantified flavor chemicals and nicotine in EC products that were manufactured by one company and purchased in four countries, and we compared flavor chemicals in authentic and counterfeit products. We identified cytotoxic refill fluids using the MTT assay and further observed their effects on cell growth using live cell imaging. We then tested authentic standards of pure flavor chemicals that may contribute to the cytotoxicity of the refill fluids. Total flavor chemical concentration was ≥ 1 mg/ml in 76% of the refill fluids and ≥ 10 mg/ml in 34%. Of 130 flavor chemicals identified, 28 were ≥ 1 mg/ml in at least one sample and 6 of these were ≥ 10 mg/ml. The total number of flavor chemicals in each product ranged from 0 - 50. For authentic refill fluids, products purchased in different countries were similar in type and concentration of flavor chemicals. However, counterfeit "Menthol" and "Bright Tobacco" products contained twice the concentration of flavor chemicals as their authentic counterparts. Identical products purchased in different countries induced similar cytotoxic responses in the MTT assay and cell growth inhibition in the live cell imaging assay. However, counterfeit "Bright Tobacco" inhibited cell growth more than its authentic counterparts. Ethyl maltol, furaneol, and eugenol contributed to toxicity. For specific authentic products, the flavor chemicals used, their concentrations, and their cytotoxicity did not vary with country of origin. Cytotoxicity was attributed to specific flavor chemicals that were present in high concentration, which often exceeded those in other consumer products. Flavor chemical concentrations in counterfeit products exceeded those in their authentic counterparts, supporting the idea that counterfeit products can contribute to VAPI. Safety of these products could be improved by regulation of their flavor chemicals.

2207 The Key Characteristics of Carcinogens

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The key characteristics of human carcinogens were recently introduced to provide a uniform approach for searching, organizing, and evaluating mechanistic evidence to support cancer hazard identification. The key characteristics comprise the properties of known human carcinogens, including their ability to be genotoxic; be immunosuppressive; or modulate receptor-mediated effects. They differ from the hallmarks of cancer, which are the properties of cancer cells. The key characteristics have been applied in evaluations of more than 50 mechanistically diverse chemicals and complex exposures classified into Groups 1, 2A, 2B, and 3 by IARC Monographs expert Working Groups since 2015. Because they are based on empirical observations of the properties associated with known carcinogens rather than an *a priori* hypothesis about a mechanism of action, the key characteristics provided an agnostic and unbiased survey of the mechanistic literature. This improved uniformity across evaluations of mechanistically diverse agents. It also improved transparency, revealing strengths as well as gaps in evidence, and highlighting mechanistic similarities and differences. However, some challenges, including in interpreting evidence on individual key characteristics, were also identified. In 2019 amendments, the IARC Monographs Preamble has adopted an approach based the key characteristics. The Preamble addresses some of the identified challenges, and also emphasizes study quality considerations in the review of evidence relevant to the key characteristics. These are important advancements to prepare for future advances in molecular research aimed at identifying the causes of human cancer, the first step in cancer prevention.

2208 Comparative Risk Assessment of Regulated vs. Unregulated Cannabis Concentrate Aerosol ("Vape") Products

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The recent epidemic of vaping-related lung injuries has focused attention on risks associated with illicit cannabis "vape pens." In a series of studies, we quantified some of the risks associated with illicit vape products, relative to regulated ones. Although regulated markets for cannabis products exist in 33 states, the unregulated, illicit market still accounts for the majority of cannabis consumed in the US. Cannabis products sold outside of regulated channels are not subject to testing for potency, impurities or contaminants. Three important differences distinguish regulated products from unregulated: the hardware, the cannabis content, and diluents. Poor manufacturing processes may introduce toxic heavy metals into cartridge components, which may leach into cannabis oils. Unsafe cultivation processes can introduce pesticides, which are concentrated during the process of extracting and distilling cannabis. Components of comparable regulated and unregulated hardware products were subjected to elemental analysis. Two common diluents -- MCT oil (sometimes used in regulated products) and Vitamin E acetate (often used in unregulated products) -- were aerosolized and tested for chemical composition. Finally, regulated and unregulated products were tested for pesticides and potency. Exposure to these substances was assessed using a new model of cannabis concentrate aerosol consumption ("vaping"). This model was based on usage data from tens of thousands of regulated cannabis users that opted-in to sharing their data were collected via a mobile app connected to a digital vaporizer. We conclude that regulated products present a lower risk to the end user than unregulated products. These results have important implications for public health policy makers, as they develop strategies to address the safety risks from illicit vapor products.

2209 Systematic Assessment of Cellular Gene Network Modulation by Valproic Acid Analogues for Biological Read-Across

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Chemical read-across is commonly evaluated without particular knowledge about the biological mechanisms leading to the observed adverse outcomes in *in vivo* studies. The integration of data showing a shared mode of action in humans will strengthen the read-across assessment. Here we used a large panel of valproic acid (VPA) analogues to include detailed mode-of-action (MoA) data as a proof-of-concept for read across. In rodents, VPA and some of its analogues cause hepatic steatosis, whereas other analogues do not. Previous studies showed a predictive value for stress pathway response activation for the hepatotoxic potential of VPA analogues. Here, we look into the transcriptomic response of HepG2 cells stimulated for 24 h by the analogues in a dose response range to be able to predict steatotic potential *in vivo*. We used TempO-Seq targeted high-throughput screening assay to assess the differential expression of more than 3000 genes, referred as the S1500+ geneset, that reflects all biological pathways. Dose response analysis revealed clustering of steatotic-positive versus steatotic-negative VPA analogues. To quantitatively define biological read-across of VPA analogues we used both pathway analysis and our in-house primary human hepatocyte (PHH) TXG-MAPr tool, a novel gene expression visualization tool based on weighted gene co-expression network analysis of the Open TG-GATEs database. VPA responses in HepG2 cells displayed high similarity in gene network modulation compared to responses in PHH. Steatotic VPA analogues demonstrated similar pathway activation. Quantitative gene network analysis allowed ranking of biological similarity of VPA analogues. Importantly, free fatty acid synthesis modulation was highly affected by all steatotic VPA analogues, but not by *in vivo* negative analogues. We defined the most representative genes that reflect steatosis responses in both HepG2 and PHH that could be generally used as markers for steatosis onset. In summary, by studying the MoA on a transcriptional level, we anticipate to support risk assessment by providing quantitative and mechanistic biological information to corroborate a robust read-across approach. *This work was part of the EU-ToxRisk project and received funding of the European Union's Horizon 2020 research and innovation program under grant agreement No 681002.*

2210 Evaluation of Risk Associated with Heavy Metals in *Carica papaya* (Pawpaw) from Three Waste Dump Sites in Rivers State, Nigeria

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The fruit of *Carica papaya* (Papaya) is popularly consumed worldwide; but in addition, the leaves and root decoction of the plant are also used for various medicinal purposes in Africa. In this study, the risk associated with heavy metals (Cr, Cd &Pb) in *Carica papaya* (CP) from three local Government refuse sites in Rivers, Nigeria, was measured and assessed. The leaf, root and fruit samples of CP were collected and transported to the laboratory for six months, at intervals of six weeks. The concentrations of the heavy metals in the samples were analysed using the Atomic Absorption Spectrophometer. The potential risk associated with consumption of these metals was evaluated in terms of Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazardous Index (HI). The EDI values (mgkg-1day1) of Cr, Cd &Pb, respectively, were as follows; for Leaf: station1 (7.82×10-4, 8.99×10-5, 9.65×10-4), station2 (7.92×10-4, 6.16×10-5, 1.10×10-3), station3 (6.83×10-4, 7.32×10-5,1.12×10-3); Root: station1 (6.19×10-4, 4.33×10-5, 1.03×10-3), station2 (3.78×10-4, 4.65×10-5, 6.57×10-4), station3 (3.99×10-4, 6.82×10-5, 8.07×10-4); and Fruit: station1 (3.26×10-4, 3.16×10-5, 7.69×10-4), station2 (3.61×10-4, 2.0×10-5, 2.70×10-4), and station3 (9.15×10-5, 3.83×10-5, 4.46×10-4). DerivedTHQ values were: Leaf, in station1 (0.52, 5.99, 0.69), station2 (0.53, 4.12, 0.79), station3 (0.46, 4.82, 0.80), for Root: station1 (0.41, 2.89, 0.74), station2 (0.25, 3.10, 0.47), station3 (0.27, 4.55, 0.58); and Fruit: station1 (0.22, 2.11, 0.55), station2 (0.24, 1.33, 0.19), station3 (0.06, 2.55, 0.32), respectively. Computed HI for Leaf in station1 (7.2), station2 (5.44) & station3 (6.08); Root, station1 (4.04), station2 (3.82), & station3 (5.4); and for Fruit, station1 (2.88), station2 (1.76) & station3 (2.93), respectively. The study revealed that Cr, Cd &Pb were bioconcentratedin CP with values higher than WHO/FAO safety limits. Therefore, consumption of *Carica papaya* plant parts may pose heavy metal health risk in the study locations.

2211 Categorical Read-Across Assessment of Cancer and Noncancer Endpoints for Amino-Dinitrotoluenes

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For some chemicals to which we are all exposed, rich data sets incorporating chronic, reproductive and developmental bioassays are available. However, most chemicals are "data-poor", hindering the identification of toxicity thresholds. 4-Amino-2,6-Dinitrotoluene (4-ADNT), and 2-Amino-4,6-Dinitrotoluene (2-ADNT), collectively, ADNTs, are members of a category of compounds known as nitroaromatics. The two ADNTs are the main human urinary metabolites of trinitrotoluene (TNT). There are no repeat-dose toxicity studies for the ADNTs, however, both are active in most genotoxicity tests. A categorical read across (RA) process was used to identify a surrogate for the derivation of a toxicity value for noncancer effects, and an RA process using several SAR tools was used in the weight of evidence analysis of carcinogenic effects. Three similarity contexts (structural, metabolic, and toxicitylike) were utilized to facilitate the final noncancer analogue chemical selection. Six structural analogues with published toxicity values were identified (TNT, 2-Methyl-5-Nitroaniline, Isopropalin, Pendimethalin and Trifuralin). Whereas analogues other than TNT do not share common metabolites, all analogues share metabolic features such as reduction of nitro-groups to amino-groups during metabolism. Toxicity profiles of the analogues in animal studies show that all produce methemoglobinemia and related blood effects. Only isopropalin failed to produce hepatic effects. This suite of toxic effects, beginning with reactive oxidant species (ROS) produced in red blood cells resulting in oxidation of hemoglobin (methemoglobin), red blood cell destruction, splenic effects from damaged RBC processing and Kupffer cell and other liver effects subsequent to ROS, and other effects from damaged RBCs are common features of organic and inorganic nitrate exposure. TNT proved to be the most appropriate analogue, in terms of structural, metabolism and toxicity. In the carcinogenicity analysis, analogues were identified through structural clustering with known carcinogens (ChemACE). ADME, genotoxicity, and carcinogenic data were evaluated and a SAR analysis was performed on the target and potential analogues for structural alerts known to be associated with carcinogenicity. Based on similarities across these data streams, we determined that ADNTs could reasonably be expected to lead to carcinogenicity. *The views expressed in this abstract are those of the author and do not necessarily reflect the views and policies of the US EPA.*

2212 Evaluation of Serum and Urine Metabolites Perturbed in Hyperglycemia Induced by Bisphenol S Exposure in Wistar Rats

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Bisphenol S (BPS) is an industrial chemical and widespread in the environment causing detrimental effects proportionally similar to its congener substance bisphenol A (BPA). Earlier studies have been focused on its estrogenic potential. Based on the available literature, we hypothesized that it may perturb the expression of endogenous metabolites in several biochemical pathways. In the present study, we have characterized perturbed metabolites in serum and urine of male Wistar rats. Experimentally, BPS was administered orally at doses of 0.05, 0.5 and 5 mg/kg bw (low, medium and high) for 90 days. The oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) showed an elevated level of glucose in exposed animals at medium and high doses, indicated the manifestation of hyperglycemia. Further, GC-MS/MS analysis identified 24 and 9 differential metabolites in serum and urine, respectively, consisted of amino acids, sugars, and organic acids. In addition, multivariate data of differential metabolites were obtained by reprocessing of GC-MS/MS data using metaboanalyst software; comprising ANOVA data of individual metabolites, principle component analysis (PCA) and partial least square discriminant analysis (PLS-DA) data. Where, variance in different groups explained in foremost two PCs with 32.1 % and 15.5 % of PC1 and PC2, respectively. Furthermore, perturbed metabolites were explored to identify the altered pathways by Kyoto Encyclopedia of Genes and Genomes (KEGG) based analysis. The result showed amino acid, glycolysis, pyruvate metabolism, and other pathways affected due to BPS exposure. Conclusively, the study showed perturbed metabolites promisingly revealing BPS exposure induce hyperglycemia.

2213 The Impact of the Seasonal Biorhythms on Hematotoxicity in Rats by Treatment of Cyclophosphamide, Methotrexate, and 5-Fluorouracil

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The same doses of xenobiotics are administered at different times of the day or seasons may be accompanied by variability in the amplitude of the toxic effects. The investigation of chronotoxicological regularity for pollutants contributes to a more accurate determination of toxicological properties, which ultimately facilitates to identify and reduce environmental health risks of chemicals. The aim was to determine chronotoxicological profile of combination the cytostatics CMF by marker parameter - count of leukocytes. 84 male *Wistar* rats aged 90 ± 5 days were used in the work. The experiment was carried out during a year, two equal randomized groups were formed every two months. Housing and feeding conditions were standard for vivarium. Experimental design: control group - solvents; the experimental group - intraperitoneally cyclophosphamide 100 mg/m^2 (once a day for two weeks), methotrexate 40 mg/m^2 and 5-fluorouracil 600 mg/m^2 on the first and eighth days. The administration time was 11:30. 24 hours after the last injection the animals were euthanized (CO_2 chamber), blood sampling with an anticoagulant (heparin) was analyzed *ex tempore* on Erma PCE-210 automated hematology analyzer (Japan). Statistical processing was based on Student's *t*-test. Administration to experimental animals a complex of cytostatics was accompanied by the expected decrease in the total number of white blood cells (WBC) - on the average of 73%. The mean annual count of WBC in control animals was 5.9 ± 0.8 x 10^9 /L. In August (the bathyphase) it decreased to 4.8 ± 0.8 × 10^9, in February (the acrophase) it increased to 6.8 ± 0.4 × 10^9 ($p<0.01$). The range of values between the extreme points was 29%. In animals of the experimental groups the average annual number of WBC was 1.6 ± 0.3 x10^9/L. The minimum was detected in August - 1.0 ± 0.2 x10^9, the maximum - in October and December - 1.8 ± 0.2 x10^9 which defines a 180% increase ($p<0.001$). The wide variability in the hematotoxic effect of the used chemicals (CMF) reveals the modulating action from seasonal biorhythms, which in modeled pathology are manifested by a larger amplitude of the body's response reactions. This circumstance should be taken into account in procedures for assessing acute and chronic toxicity of testing substances to increase the veracity of the information received.

2214 Proposal for a Health-Based Occupational Exposure Limit for Trichloramine by the Nordic Expert Group

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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemica (NEG) is a collaboration for production of criteria documents on chemicals as basis for setting occupational exposure limits (OELs) in the Nordic countries (1). A criteria document on inorganic chloramines, including a health-based OEL proposal for trichloramine, was recently published (2). Trichloramine is formed as one of several disinfection byproducts when chlorine reacts with nitrogen containing compounds. Trichloramine is immiscible with water and evaporates easily from water into air. Trichloramine may aggravate asthma symptoms in individuals with existing asthma, however, NEG concludes that the risk of developing asthma following long-term exposure to trichloramine cannot be evaluated at present. No data on genotoxic, carcinogenic, reproductive or developmental effects were located. Based on several studies on indoor swimming pool workers the critical effect is judged to be irritation, starting at approximately 0.4 mg/m^3 (stationary measurements). Applying assessment factors (AF) to account for extrapolation from LOAEC to NOAEC (AF 2) and from stationary to personal measurements (AF 2), NEG recommends a health-based OEL of 0.1 mg/m^3 (8-h TWA), corresponding to 0.2 mg/m^3 for stationary measurements in swimming pool facilities. No short-term exposure limit (STEL) is recommended. For comparison, the World Health Organization (WHO) in 2006 recommended a maximum trichloramine level of 0.5 mg/m^3 as a comfort value in indoor swimming pool air (3). *References: (1) https://www.av.se/en/the-nordic-expert-group/. (2) Wastensson G, Eriksson K (2019) Arbete och Hälsa 53(2):1-110. http://hdl.handle.net/2077/61724. (3) WHO (2006) Guidelines for safe recreational water environments. Vol. 2. Swimming pools and similar environments. https://www.who.int/water_sanitation_health/publications/safe-recreational-water-guidelines-2/en/.*

2215 An Integrated Approach of Dietary Exposure and Physiologically Motivated Toxicokinetic Modeling for Probabilistic Risk Assessment of Nitrate and Nitrite through Vegetable Consumption

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Food safety of nitrate and nitrite in vegetables has been a concern of many regulatory bodies and researchers. This study aimed to determine the concentrations of nitrate and nitrite in vegetables and to estimate the potential dietary risk of nitrate and nitrite due to vegetable consumption based on internal dose in a probabilistic manner by integrating dietary exposure assessment with physiologically-motivated toxicokinetic (TK) modeling. In this study, the concentrations of nitrate and nitrite in 21 vegetables were determined based on the massive consumption database from the Nutrition and Health Survey in Taiwan (NAHSIT) covering all ages. We optimized a previously established TK model, validated our new model using human data, and incorporated Monte Carlo simulations to account for variability across human populations for different age groups. High levels of nitrate were detected in leafy vegetables with the means ranging from 545.33 to 1641.17 mg/kg. Nitrite contents of vegetables were generally low with the means ranging from 1.27 mg/kg in potato to 8.20 mg/kg in sweet potato vines. The major sources contributing to the average nitrate and nitrite exposures across age populations were cruciferous leaf vegetables and cabbage. The assessment results were found to be different between based on external vs. internal dose, suggesting that it is critical to include the kinetic process of the endogenous formation of nitrite into risk assessment of nitrate and nitrite. Our results suggest that nitrate and nitrite exposure from vegetables is unlikely to result in appreciable safety risk for most age populations, but may be a potential concern for preschoolers. This is the first study integrating dietary exposure assessment, a TK model in humans, with probabilistic approach to predict the internal dose for estimating the dietary risk caused by nitrate and nitrite in vegetables. Our method can be applied to obtain more reliable exposure estimates and to improve risk assessment of nitrate and nitrite.

2216 *In Vitro* and *In Vivo* Identification of a Soluble Epoxide Hydrolase (sEH) Metabolite, 14,15-DHET, as an Early Doxorubicin (DOX) Cardiotoxicity Biomarker and Screening of sEH Inhibitors for Medical Intervention

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Approximately 610,000 cancer-related deaths were estimated for 2018 in US. Chemo therapy is the most common treatment given to cancer patients. However, the treatment may induce cardiac dysfunction. Detection of early biomarkers that predicts cardiac dysfunction offers opportunities to adjust an individual's treatment during chemo therapy. Attenuation of the conversion of epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs) by inhibiting the activity of sEH has been tested as a therapeutic approach for treating cardiovascular diseases. The aims of this study are to find whether 14,15-DHET is an early biomarker to predict DOX-induced cardiotoxicity using cell media of DOX-treated H9c2 rat cardiomyocytes and serum of DOX-treated female Sprague-Dawley rats and to screen inhibitors, which may prevent the sEH-dependent cardiotoxicity. H9c2 cells were incubated with and without 1 μM DOX for 2 hr and cells and media were collected after 2, 6 and 26 hr recovery periods. H9c2 hypertrophy (cell area and BNP expression) and 14,15-DHET levels in cell media by ELISA were assessed. Cellular hypertrophy was detected only after 6 and 24 hr of recovery period following DOX treatment and not after 2 hours. Levels of 14,15-DHET increased after 2 hr recovery period prior to detection of cellular hypertrophy and 14,15-DHET remained elevated after 6 and 24 hours. sEH expression in the cells was confirmed by 2.6- and 4.5-fold elevated 14,15-DHET levels in cell media after treatment with and without 1 and 5 μM EET, respectively. Honokiol, a component of magnolia bark, and AUDA, a synthetic sEH inhibitor, inhibited sEH activity in a reconstituted system with recombinant sEH. The result obtained with H9c2 cells was confirmed using a rat serum with and without DOX treatment (3 mg/kg /week for 2 weeks) (no recovery group) and after a 2-week recovery period (recovery group). After two weeks of DOX treatment, when cardiotoxicity was not detected, levels of 14,15-DHET were increased compared to the control group. The results suggest that 14,15-DHET is an early biomarker to predict DOX-induced cardiotoxicity. Early diagnosis of patients to predict cardiac dysfunction is necessary to adjust the anti-cancer drug treatment protocol. Moreover, inhibition of sEH may ameliorate the chemotherapy-induced cardiotoxicity. *Supported by NHLBI SBIR Phase I Contract HHSN261201600028C.*

2217 Dihydroxyacetone Exposure Alters NAD$^+$/NADH-Inducing Mitochondrial Stress and Autophagy in HEK293T Cells

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Dihydroxyacetone exposure alters NAD$^+$/NADH inducing mitochondrial stress and autophagy in HEK293T cells. Dihydroxyacetone (DHA) is the triose precursor to dihydroxyacetone phosphate (DHAP). DHA is used as the active ingredient in sunless tanning products (STPs) where its reaction with amines in the skin produces the STPs browning effect. DHA is also a by-product generated by the heat-driven oxidation of glycerol, a component of e-cigarette flavoring. Human exposure to DHA has been increasing as the popularity of STPs and electronic cigarettes has grown. Little is known about systemic effects of exogenous DHA exposure through absorption or inhalation. This present study uses HEK 293T cells in investigation of systemic effects of exogenous DHA exposure. HEK293T cells were sensitive to consumer-relevant doses of DHA, with an IC_{50} value of 2.4 ± 0.3 mM. In order to evaluate acute exposure effects, we have kept our dosing concentration at the IC_{90} value of 5 mM DHA. Flow cytometry and propidium iodide staining revealed cell cycle arrest, with the G2/M fraction doubling at 48 h, then increasing out to 72 h. TMRE staining indicated reduced mitochondrial function over time, while assays for ATP levels showed ATP reduction beginning at 24 h. In addition to reduced function, mitochondria also exhibited morphological changes viewed by super resolution microscopy. Auto-fluorescent imaging and NAD+ biosensors revealed an imbalance in the redox cofactors NAD+/NADH within 24 h of exposure. Assays for levels of reduced and oxidized glutathione showed a decrease in reduced glutathione and an increase in oxidized glutathione, suggesting reduced cellular capacity for handling reactive species. A decrease in the ratio of LC3B I/II at 48 h, along with increases in SIRT1 expression over time, indicated autophagy as a driver of cell death. Despite DHA's ability to be converted to DHAP and integrated into metabolic pathways, the metabolic dysfunction and starvation responses observed in the HEK293T cells indicate that DHA does not readily contribute to the energetic pool in these cells.

2218 Dietary Arsenic Risk Assessment and Risk Management

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Regulatory limits in dietary arsenic have so far focused on specific foods without adequate consideration of overall dietary intake. Concerns about dietary inorganic arsenic (iAs) have focused on rice, and especially on rice-based foods for infants; however, other food groups typically contribute more to daily intake, with vegetables and fruits contributing over 50% of the daily intake for a typical American compared with less than 20% from rice. Beer, wine and other grains are estimated to contribute another 20% of intake. Due to the lack of reliable data on iAs concentrations in most foods, daily iAs intake estimates are quite variable. For Americans, the mean iAs intake from food has been estimated to be 0.05 µg/kg b.w./day. The European Food Safety Authority estimated higher daily intakes, with mean adult intakes ranging from 0.09 to 0.38 µg/kg and 95th percentile dietary intakes ranging from 0.14 to 0.64 µg/kg; however, these estimates include drinking water intake and assumed iAs was 70% of total arsenic for most foods. These intake estimates also do not consider cooking losses and reduced bioavailability from some foods. Most recent studies show a correlation between urine arsenic and high iAs foods; however, epidemiology studies generally have not had adequate exposure data to support assessment of impacts on disease burden. Using the current USEPA slope factor, average dietary iAs would be associated with unacceptable increases in cancer risk, and several schemes are being proposed to reduce arsenic exposure by intervening at multiple levels in the food supply chain. Many of the proposed interventions could have significant implications for the food supply of people in developing countries who rely heavily on rice. It has recently been proposed that cancer induction by inorganic arsenic has a threshold drinking water concentration of 100 ug/L (with a range of 50-150 µg/L). For a 70 kg adult consuming 2 L/day at 100 µg/L, the safe daily intake would be 2.9 µg/kg. If drinking water only contains 10 µg/L, a dietary intake of 1.7 µg/kg would be safe. This estimate exceeds high end dietary intake estimates by a substantial margin. If this threshold is accurate, iAs should not be a source of health risk for people without elevated drinking water arsenic. Exceptions might be intakes for infants consuming largely rice-based diets and populations consuming certain seaweeds with very high iAs. Given potential disruptions to the food supply and possible associated adverse effects from attempts to control iAs exposure, it is imperative that arsenic risks be accurately characterized.

2219 Filling the Gap between Regulation and Public Health Risk Characterization: Case Study in Lead ALE Sites in Michigan

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Lead is one of the most ubiquitous toxic substances and can be found in all parts of the environment including air, soil, and water. Lead in pipes can enter drinking water when the pipes corrode or break down. US EPA established the Lead and Copper Rule (LCR) in 1991 to monitor and reduce lead in drinking water. LCR requires public water supplies to sample water and test lead at customer taps. A lead action level exceedance will trigger required actions which includes adjusting corrosion control and delivering customer notice. In June 2018, Michigan made a step forward and adopted the country's most strict LCR to further reduce exposure to lead from drinking water. Along the protections in the Michigan's revised LCR, Michigan Department of Health and Human Services (MDHHS) reaches out to residents and conducts sequential samplings. Results from sequential sampling are used to identify lead sources in plumbing and to adjust flushing time recommendations. The results provide more information on the lead exposure levels for exposure assessment and risk characterization in different scenarios. The estimated levels were then entered into US EPA IEUBK model to estimate the health risk. It is indicated that the action of flushing for right amount of time would effectively reduce lead exposure from drinking water and lower the probability for children to have an elevated blood lead level (>5 µg/dL). MDHHS is also engaged in various types of public health actions to protect people against exposure to other lead sources.

2220 Risk Assessment of the Flame Retardant Chemical Tetrabromobisphenol A (TBBPA)

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TBPPA (79-94-7) is the highest production volume organohalogen flame retardant (OFR) chemical. The purpose of this study is to conduct a preliminary risk assessment for TBBPA by using the currently available exposure data and key toxicity studies. TBBPA has been detected in indoor dust and air, and in human tissues. The TBBPA concentration in plastic enclosures of TVs and computer monitors ranges from 11 percent to 27 percent, based on XRF, GC-MS, and LC-MS analysis of discarded products. The NTP 2-year bioassay indicates TBBPA can cause cancer in animals; the unit cancer risk of TBBPA is 0.002 $(mg/kg\text{-}d)^{-1}$. based on uterine cancer. TBBPA was consistently reported to inhibit thyroid function in both reproductive and developmental and repeated-dose toxicity studies. The acceptable daily intake is 0.6 mg/kg-d, based on the reduction of T4 thyroid hormone levels. Human biomonitoring data and environmental monitoring data from indoor dust, indoor air, diet, and hand-wipes were used to estimate TBBPA exposures. Estimated TBBPA average daily exposures ranged from 1 $x10^{-6}$ to 2.4 $x10^{-2}$ mg/kg-d. This results in hazard indices of 2 $x10^{-6}$ to 4 $x10^{-2}$ and lifetime excess cancer risks of 2.1 $x10^{-9}$ to 5 $x10^{-5}$. TBBPA exposures derived from environmental monitoring overlap with, but do not fully explain, the range of data observed in human biomonitoring. For example, one human biomonitoring study conducted with 140 volunteers in south China suggests much higher exposures and risks than modeled exposures. In summary, these analysis indicate more research, including human biomonitoring studies for the US population, and migration studies for TBBPA containing consumer products, are needed to better assess the health effects of TBBPA.

2221 Aflatoxin-Related Immunotoxicity: A Dose-Response Assessment

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Aflatoxins are toxic compounds produced by the fungi *Aspergillus flavus* and *A. parasiticus*. In warm climates, these fungi frequently contaminate food commodities such as maize and peanuts. In many parts of the developing world, especially in sub-Saharan Africa, people are co-exposed to dietary aflatoxin and multiple infectious agents in food, water, and the environment. There is evidence suggesting that aflatoxin exposure may lead to immune dysfunction, which could increase infectious disease risk in vulnerable populations. Our aim was to conduct a dose-response assessment on a non-carcinogenic endpoint of aflatoxin - immunological effects - beginning with a review of human and animal studies on the association between aflatoxin exposure and immune system impairment. We sought to determine a non-carcinogenic tolerable daily intake (TDI) of aflatoxin, based on the existing data surrounding aflatoxin and immunological effects. In our literature search on PubMed and Google scholar databases, human, animal, and *in vitro* studies were considered relevant if they included aflatoxin exposure and measurement of at least one marker of immune function. Reference lists of the included studies were also screened to identify additional relevant studies. For the dose response assessment, we found several mammalian studies with sufficient dose-response data to derive dose-response curves and benchmark doses. We selected one pig study (Meissonier et al. 2008), in which 3-week-old weaned pigs received a control diet or diets contaminated with 385, 867 or 1807 µg pure AFB_1/kg feed, and the pro-inflammatory cytokine IL-6 was measured. We generated a dose response curve and determined the benchmark dose lower confidence limit (BMDL). The BMDL was divided by the product of uncertainty factors (UF) to determine the TDI. We have assumed two different UFs for interspecies extrapolation: 2 and 10 (as this study was done in pigs) and found TDI_{20} = 4.65 µg/kg bw/day and TDI_{100} ~ 1 µg/kg bw/day. The latter TDI is identical to that for fumonisin (1 µg/kg bw/day), estimated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2011. There is considerable evidence that aflatoxin exposure alters immunological markers. As aflatoxin is a genotoxic carcinogen, international risk assessment bodies such as JECFA have not established a TDI for aflatoxin. Aflatoxin exposure data exist in the literature, upon which the non-carcinogenic risk of aflatoxin can be assessed in the future.

2222 Risk Assessment Studies of the Impact of Occupational Exposure of Pharmaceutical Workers in Baddi on the Development of Antimicrobial Drug Resistance

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Pharmaceutical workers involved with the production of antimicrobial drugs are exposed to various antimicrobial chemicals in different steps of manufacturing such as grinding, sieving, compression, granulation, mixing and filling. These exposures may lead to the development of multidrug resistance (MDR) in bacteria. Scientific reports on the occupational health hazard of pharmaceutical workers involved in manufacturing antibiotics are scarce. The present study aimed to compare the degree of bacterial resistance in pharmaceutical workers in Baddi, India to that of individuals not involved in the pharmaceutical field. Twenty male workers from five local pharmaceutical companies and twenty male subjects not involved in the pharmaceutical field (non-pharmaceutical subjects) were randomly selected. Nasal fluid, mucus/cough and stool specimens were collected from each subject and were cultured separately at 37°C for 24 hours to obtain bacterial growth. The cultured species were then identified, isolated and subjected to microbial sensitivity testing against 18 different antibiotics from eight different groups by the disk diffusion method. *Staphylococcus* spp., *Pseudomonas* spp. and *Escherichia coli* were identified and isolated from the culture of nasal fluids, mucuses and stools, respectively. All the isolated species of bacteria exhibited significant enhancement of the degree of MDR in pharmaceutical workers compared with non-pharmaceutical subjects. Workers with a longer working history had greater degree of antibiotic resistance and vice versa. It can be certainly considered that the exposure of pharmaceutical workers to antibiotic agents resulted in a high incidence of multidrug resistance. Effective steps should be taken to minimize inherent exposure of pharmaceutical workers to antibiotics during work to prevent antimicrobial drug resistance. Further a computational model/QSAR can be developed for antibiotic for multidrug resistance.

2223 Derivation of an Oral Reference Dose for Methyl *tert*-butyl Ether (MTBE) with a Comparative Analysis of Toxicokinetic Data in Rats and Humans

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In chronic two-year drinking water studies, exposure to methyl *tert*-butyl ether (MTBE) in rats is associated with a wide range of kidney effects, including increased severity of chronic progressive nephropathy and increased incidence of kidney tumors. Due in part to its historical use as a gasoline additive, exposure to MTBE via drinking water can also occur in humans as a result of contaminated wells and drinking water supplies. The aim of the current research was to identify an oral reference dose for MTBE and to consider whether the available toxicokinetic data in rats and humans allow for the use of a chemical-specific adjustment factor (CSAF) for extrapolation of the point of departure (POD) from a rat-specific dose to a human-equivalent dose. As interpretation of the kidney effects in male rats are confounded by a male rat-specific mode of action (alpha-2μ globulin nephropathy), the critical effect was identified from the most sensitive kidney effect in female rats: increased relative kidney weight with histopathological correlates of increased incidence of renal tubular hyperplasia, papilla mineralization, and pelvis mineralization. The available toxicokinetic data were subsequently reviewed to determine the feasibility of deriving a CSAF for interspecies extrapolation. In rats, plasma area under the curve (AUC) values for both MTBE and its primary metabolite *tert*-butyl alcohol (TBA) have been predicted from a physiologically-based pharmacokinetic (PBPK) model for a wide range of doses (~50 - 1,200 mg/kg-day) based on a 91-day exposure. Conversely in human subjects, plasma AUC values were measured in subjects exposed singly to only one comparatively low dose level (0.15 mg/kg). Recognizing that the differences in exposure level and duration associated with the predicted rat AUC values and the measured human AUC values preclude valid comparisons, we concluded that the available toxicokinetic data are insufficient to derive a CSAF. Instead, the default US EPA dosimetric adjustment method was utilized for the interspecies extrapolation. A POD of 156 mg/kg-day was derived from the lower confidence limit on the human-equivalent benchmark dose ($BMDL_{HED}$) associated with the critical effect, using a benchmark response rate of one standard deviation above the control mean. Based on an uncertainty factor of 30 (3x for intraspecies differences, 10x for interspecies differences), the reference dose is 5 mg/kg-day. Expansion of the existing rat PBPK model to allow for predicting relevant TK parameters in humans at similar dose levels as rats will be useful for refinement of the oral reference dose for MTBE.

2224 QIVIVE (Quantitative *In Vitro* to *In Vivo* Extrapolation) in Practice: A Comparative Assessment of Emerging and Conventional Risk Assessment and Associated Interpretive Opportunities

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The wealth of *in vitro* data that have been generated as part of the Tox21 and ToxCast initiatives or that may be generated in the future using New Approach Methodologies provides an opportunity to refine the science of risk assessment. Such an approach fills data gaps, reduces animal usage, provides human relevant data that increases confidence and reduces uncertainty compared to conventional approaches, and reduces the time and costs necessary to generate safety data, thus advancing opportunities to innovate while effectively protecting public health. Ideally within this process, adverse outcome pathways (AOPs) that identify key events necessary for adverse apical outcomes are aligned to a battery of *in vitro* assays. Reverse dosimetry, or QIVIVE, of the assay activity concentration at cutoff (ACC) using compound-specific parameters within pharmacokinetic (PK) models provides an estimated human daily equivalent administered dose (EAD) that would result in a plasma concentration corresponding to the *in vitro* ACC, the "lowest effect level" for assay activity. In this study, oral EADs for two suspected estrogen receptor agonists, *p-tert*-butylphenol (CAS# 98-54-4) and benzophenone (CAS# 119-61-9) were derived from active *in vitro* assays and compared to points of departure (PODs) from the available two-generation reproductive toxicity studies in rats. One compartment (1C) and three-compartment (3C) PK models were applied to estimate oral EADs using the NTP Integrated Chemical Environment (ICE) tool and the median ACC_{ER}. For *p-tert*-butylphenol, EADs were estimated as 69 mg/kg-day (1C) and 2.2 mg/kg-day (3C, 100 days). In comparison to the two-generation study with a human equivalent LOAEL of 48 mg/kg-day, use of the 1C model provides a comparable POD; however, the apical endpoints were not consistent with estrogen agonism. For benzophenone, EADs were estimated as 85 mg/kg-day (1C) and 5.6 mg/kg-day (3C, 100 days), where the 1C EAD was comparable to the human equivalent NOEL of 38 mg/kg-day (the highest dose tested) from the two-generation study in rats; however, evidence for estrogenic effects was not observed in the two-generation study. Further evaluation of interpretive opportunities for the assays, including consideration of thresholds of adversity, apical endpoints, components of the PK models, and the value of reference chemicals, are presented.

2225 Notification Level Recommendations for Perfluorooctanoic Acid and Perfluorooctane Sulfonate in California Drinking Water

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Notification levels (NL) are non-regulatory health-based advisory levels for chemical contaminants in California drinking water that do not have regulatory standards or Maximum Contaminant Levels. NLs of 5.1 parts per trillion (ppt) for perfluorooctanoic acid (PFOA) and 6.5 ppt for perfluorooctane sulfonate (PFOS) were set by the California State Water Resources Control Board (SWRCB), based on recommendations by the Office of Environmental Health Hazard Assessment (OEHHA). OEHHA conducted a focused evaluation of recent animal and human toxicity studies of PFOA and PFOS, emphasizing known hazards (hepatotoxicity, immunotoxicity, reproductive toxicity, thyroid toxicity, and carcinogenicity). Epidemiology studies were evaluated and observed to support hazard evaluations but not included in the final dose response assessment because there were no identified studies suitable for point of departure determination. Using OEHHA's risk assessment methodology and human clearance values derived by the US EPA, OEHHA developed cancer reference levels (RL) of 0.1 ppt for PFOA based on increased incidences of pancreatic and hepatic tumors in male rats exposed orally for 2 years, and 0.4 ppt for PFOS based on increased incidences of hepatic tumors in male rats administered PFOS in the diet for 2 years. Cancer RLs represent the concentration of the chemicals in drinking water estimated to not pose more than a one in one million cancer risk over a lifetime. RLs for non-cancer endpoints were also developed: 2 ppt for PFOA based on biomarkers of liver toxicity in female mice; and 7 ppt for PFOS based on immunotoxicity in male mice. Because the cancer RLs are below quantifiable levels of detection in water, OEHHA recommended setting the NLs at the lowest levels at which PFOA and PFOS can be reliably detected using available and appropriate techniques.

2226 Risk Assessment of the Drinking Water Treatment Chemical 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC)

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Use of 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTC) as a corrosion and scale inhibitor in drinking water treatment may result in PBTC residual of drinking water. Dermal exposures to PBTC also occur predominantly through the use of industrial and consumer cleaning products containing PBTC. An oral risk assessment was undertaken to establish allowable drinking water levels. Due to inadequate human health effects data, hazard identification relied upon laboratory animal data. No adverse effects were seen after dietary PBTC exposure up to the highest tested doses of 461 and 513 mg/kg-day for male and female Wistar rats, respectively, after 90 days, the longest-term data identified. High LD_{50} values > 10,000 mg/kg also suggest a low toxicity profile for PBTC. The available *in vivo* and *in vitro* genetic toxicity studies for PBTC suggest low concern for genotoxicity. No reproductive studies were identified and the one developmental gavage study in Wistar rats showed no adverse effects up to the highest tested dose of 1000 mg/kg-day. Since the subchronic key study was unpublished and secondary sources poorly characterized the test substance, the NOAEL was adjusted based upon the commercial percentage of PBTC typically used (~40%) to an adjusted human equivalent $NOAEL_{HED}$ of 42 mg/kg-day as the point of departure. A 3x rather than default 10x study duration uncertainty factor is supported by the free-standing NOAEL combined with the high-water solubility and the apparent rapid elimination which is supported by the low toxicity. The lack of tested analog or surrogate compounds limited the ability to apply read-across to fill in data gaps. Using a 1000x composite uncertainty factor (3x interspecies, 10x intraspecies, 1x LOAEL to NOAEL, 3x study duration, 10x database), an oral RfD of 0.04 mg/kg-day was determined for PBTC resulting in a Total Allowable Concentration in drinking water of 1 mg/L assuming 0.032 L/kg-day adult water intake and a 80% relative source contribution to account for other non-drinking water routes of exposure.

2227 Evaluation of the Carcinogenic Potential of Baclofen after Dose Titration in rasH2 Mice

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Baclofen, a selective gamma-aminobutyric acid-B receptor agonist, has emerged as a promising drug for long term treatment of alcohol dependence. We evaluated the carcinogenic potential of Baclofen in a 26-week study by the oral route (gavage) in male and female CB6F1-TgrasH2 transgenic mice at dose levels of 45, 90 and 180 mg/kg/day. Dose titration of Baclofen is necessary before chronic treatment, so the animals were given three increasing dose levels of Baclofen for 5 days each before starting the 26 week treatment period. Vehicle control (0.5% methylcellulose aqueous solution) and positive control (N-methyl-N-nitrosurea) groups were included in the study. Toxicokinetics were evaluated concomitantly in non-transgenic CB6F1-nonTgrasH2 (wild type) satellite mice. Health status, central nervous system activity, food consumption, body weight and the possible onset of masses were monitored. Designated organs were microscopically examined. Survival rates were not affected by treatment. Maximum plasma Baclofen concentrations were reached at 0.5h on day 1 and at 0.75h on day 25, and no accumulation was observed on day 25. Clinical signs, body weight loss and low food consumption were noted in the high dose group on the first 3 days at the highest titration dose level and during the first 2 weeks of the treatment period; values were similar to controls from week 3, thus demonstrating habituation to Baclofen. There were no treatment related palpable masses or neoplastic findings after Baclofen administration up to week 26. Treatment related non-neoplastic findings were limited to non-adverse increased lymphoid atrophy in the thymus (females at 45 or 90mg/kg; males and females at 180 mg/kg). In conclusion, based on the conditions of this study, with titrated dose levels prior to the beginning of the study, Baclofen was considered to be non-carcinogenic after oral administration in CB6F1-TgrasH2 transgenic mice.

2228 Risk Assessment of the Phosphorothioate Antisense Oligonucleotide Drug Class and Thrombocytopenia

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Thrombocytopenia serious adverse events, including fatality, have occurred in clinical trials with systemically administered phosphorothioate (PS) antisense oligonucleotide (ASO) therapeutics; however, class-relatedness and predictability have remained unclear and a topic of debate. To address these uncertainties, all systemically-administered PS ASOs with past and ongoing clinical trials in the US were examined for clinical thrombocytopenia events and dose margins were determined at the no observed adverse effect level (NOAEL) for thrombocytopenia in non-human primate (NHP) studies. The data indicate that thrombocytopenia is a class-related effect that can be predicted by the NHP NOAEL for thrombocytopenia, with generational trends on safety margins. Based on the current available clinical and nonclinical data, a safety margin threshold for thrombocytopenia risk is proposed, which may be used to guide risk hazard assessments and clinical monitoring programs.

2229 Adequacy of Available Data to Derive Provisional Oral Toxicity Values for 2-nitropropane

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The adequacy of epidemiologic, toxicologic, and mechanistic data was assessed for deriving a provisional oral reference dose (RfD) for 2-nitropropane (2-NP). No oral subchronic or reproductive/developmental studies are available for 2-NP, and the chronic oral database is limited to a single 16-week gavage study in rats with a single dose level and limited reporting on nonneoplastic endpoints. Available short-term and acute oral studies include 14- to 28-day studies in rats that only evaluated clinical signs, body weight, and liver endpoints and 1- to 14-day studies in rats that only evaluated renal and/or liver endpoints. These studies demonstrate toxicity of 2-NP to the liver, but effects on other organs are unknown due to a lack of relevant information. Data from inhalation and injection studies that investigated a wider range of endpoints, however, clearly identify the liver as the most sensitive toxicity target of 2-NP. Supporting mechanistic studies suggest that hepatic damage is secondary to oxidative stress due to generation of reactive intermediates during denitrification of 2-NP in the liver (e.g., N-nitro compounds and oxygen radicals), and genotoxicity studies provide strong evidence for oxidative DNA damage in the liver following exposure to 2-NP. Therefore, while available oral studies are too limited in scope and/or duration to support derivation of provisional RfDs for 2-NP, the data are adequate for derivation of a screening subchronic p-RfD. The 4-week oral study provides sufficient data on several liver endpoints to perform dose-response analysis. However, this less-than-subchronic study is considered inadequate to develop a screening chronic p-RfD for 2-NP, because it is not known if the critical effect observed in the short-term study will increase in severity or provide a more sensitive basis for dose-response analysis following longer treatment duration. While there are no longer-term oral data available, inhalation data suggest that sensitivity to hepatic toxicity may increase with exposure durations >6 months. Ultimately when evaluating the adequacy of a database for deriving reference values, it is crucial to consider various lines of evidence. *The views expressed are those of the authors and do not necessarily reflect the views and policies of the US EPA.*

2230 Oral Risk Assessment of Polyethylene Glycol: Low Molecular Weight Compound as Conservative Analog for Class

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The general population may be orally exposed to polyethylene glycol (PEG) polymers via ingestion of treated drinking water due to its use as a formulation ingredient in drinking water treatment chemicals. Other commercial applications that may result in human exposure to PEG include direct or indirect food-contact uses, contact with consumer products, direct medical treatment, or occupational exposures. The dataset encompasses PEG compounds with molecular weights (MW) ranging from 200 to greater than 100,000. A low MW compound, PEG-400, was selected as a conservative read-across analog for the class-based evaluation. Collectively, studies in both humans and animals indicate that absorption of PEG and polymer MW are inversely related. Lower MW polymers, such as PEG-400, may be orally absorbed in contrast to higher MW polymers which undergo little to no absorption. Polymers of lower MW may also undergo partial metabolism, potentially via alcohol dehydrogenase,

resulting in the generation of diacid and hydroxyacid metabolites. It has been suggested that this *in vitro* oxidation of PEG into carboxylic acid metabolites is the most probable pathway for polymer degradation. Due to the anticipated low bioavailability and lack of metabolism of higher MW PEG compounds, toxicity data on a low MW compound, PEG-400, was used to derive an oral reference dose (RfD) for the class. The RfD is based on a NOAEL of 1128 mg/kg-day identified from a 13-week gavage study in Fischer 344 rats given PEG-400. Clinical signs suggestive of altered renal function, such as increased urinary protein, bilirubin, and NAG, were observed at the LOAEL of 2820 mg/kg-day. The potential osmotic effects of PEG itself, or secondary to the very high bolus dose in general, may ultimately contribute to the renal effects seen in the identified animal studies, rather than inherent hazard potential. A comparable NOAEL from chronic exposure suggests a lack of temporal progression of the critical effects or their potency. Using a 100x uncertainty factor to account for (10x) intraspecies and (3x) interspecies variability, as well as (3x) database deficiencies that consist of a lack of standardized developmental toxicity data in a rodent and non-rodent species, an RfD of 3 mg/kg-day was determined for PEG compounds. This corresponds to a Total Allowable Concentration of 20 mg/L in drinking water.

2231 Rethinking Carcinogenicity Assessment for Agrochemicals

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The two-year cancer bioassay in rodents has been considered the gold standard to assess carcinogenicity for chemicals across multiple sectors including agrochemical industry. Cancer bioassays are known to be animal and resource intense studies using at least 400 animals per study. However, the need to conduct the bioassay as a default data requirement has been questioned over the years with increased understanding of biology and cancer pathogenesis and availability of other appropriate methods to obtain information to determine the carcinogenicity potential. Specifically, with the limitations presented by the rodent cancer bioassay because of both qualitative and quantitative differences in cancer causing mechanisms, age-dependent windows of susceptibility, and target organ susceptibility. Scientific advancements leading to better technology and efficient ways to evaluate the mechanism of actions could offer alternate testing strategies for carcinogenicity evaluation. This shift in thought process of rethinking carcinogenicity initiated an effort within the agrochemical sector to conduct a retrospective analysis assessing all the data available to determine carcinogenicity potential for pesticides. As part of this effort, Corteva Agriscience™ has been conducting weight of evidence analysis for their chemistries. The weight of evidence analysis conducted on two synthetic auxin herbicides will be presented as case studies 1) florpyrauxifen-benzyl and 2) halauxifen-methyl. In this retrospective analysis, chemical properties (pH, P_{ow}, and solubility), metabolism (ADME and Toxicokinetics), and toxicity (acute, short term, genotoxicity, reproductive, endocrine, and immunotoxicity) for any indication of carcinogenic potential were considered. The outcome of this retrospective analysis was then compared to the results of the available cancer bioassays for the respective active ingredients to determine the predictivity and identify any gaps. Collectively, this exercise demonstrated that available data for these two herbicides including short term testing can be predictive of their carcinogenicity potential and thus conduct of cancer bioassays did not add scientific value to human health risk assessment. Thus, emphasizing that carcinogenicity evaluation should be based on a weight of evidence analysis considering all the lines of evidence instead of a default approach of conducting rodent cancer bioassay on every pesticide.

2232 Oral Reference Dose for Non-Essential Elements in Drinking Water: Dietary Impact on the Relative Source Contribution

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Establishing oral reference doses (RfDs) for naturally occurring elements with high dietary and water intakes is difficult. Case in point, Rubidium (Rb), found naturally in the environment as the 16th most abundant element has a general population exposure that can occur via leaching from cement materials with potable water-contact applications. The primary route of human exposure occurs naturally in the diet. Evaluation of Rb is complicated due to 1) human and animal data confounded by baseline levels of Rb in the diet, 2) similarity of biological action with potassium (K) in the body, and 3) lack of guideline toxicity studies. Dietary Rb ingestion in a typical adult is estimated at 1 to 5 mg/day or 14 to 71 µg/kg-day, while in children ingestion is estimated at 2.34 mg/day (or 78 µg/kg-day) with milk intake accounting for 50% of dietary Rb and other food sources contributing the balance. Rb is a close analog to K and both elements exert similar biological effects, such as Rb and K are interchangeable substrates for Na^{+}/K^{+} ATPase dependent transport in the cell membrane and action potentials. As a former therapy for clinical depression, the mechanism of action involves decreasing the resting potential of the neuron and thus increasing the firing rate and the release of norepinephrine for activating postsynaptic receptors. Recognizing a paucity of data on the genetic or long-term effects of Rb in humans or laboratory animals, Rb is not expected to be DNA-reactive, bioaccumulate, or pose a concern for long-term health effects. The critical effects of Rb are an increase in sensory stimulation in animals and elevated mood in humans. Antidepressant effects in human clinical trials suggest a therapeutic minimal dose of 5 mg/kg-day; therefore, a LOAEL of 5 mg/kg-day was selected as the point of departure. A total uncertainty factor of 300 accounted for uncertainty due to intraspecies (3x), LOAEL to NOAEL (3x), study duration (3x), and an incomplete database (10x). Therefore, the RfD is 0.02 mg/kg-day. The total allowable concentration in water of 500 µg/L assumed a 70 kg adult, and a 2 Liter/day intake as well as a relative source contribution (RSC) of 0.8. The RSC of 0.8 is the EPA's ceiling level and was justified based on the background level of Rb in the patient population via the diet prior to treatment.

2233 Using GreenScreen Benchmark and Sub-Benchmark Scores to Identify Safer Plasticizers

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The GreenScreen® for Safer Chemicals is a hazard assessment framework that assesses chemicals against 18 human health, environmental, and physical hazard endpoints, and based on hazard combinations, assigns a benchmark (BM) score of 1-4, with 1 being most hazardous and 4 being least hazardous. GreenScreen® hazard assessments for 61 plasticizers in the ToxFMD® Screened Chemistry Library were compared to identify safer alternatives and to differentiate among specific hazards. Of the 61 plasticizers, 18% were BM-1 ("Avoid - Chemicals of High Concern"), 49% were BM-2 ("Use but Search for Safer Substitutes"), 15% were BM-3 ("Use but Still Opportunity for Improvement"), and 10% were BM-3DG, due to a data gap (DG) that prevents a BM-4 score (comprising epoxidized fatty esters, glycols, and terephthalates). Across the 61 plasticizers, 59% have a data gap for endocrine activity, 44% for respiratory sensitization, and 34% for neurotoxicity. Because almost half of the 61 plasticizers are scored as BM-2s, the 30 BM-2 plasticizers were studied to see if differentiation in hazard level is feasible. The GreenScreen® framework assigns BM-2 scores due to 7 sub-benchmark scores (a-g). Of the 30 BM-2s, 83% are BM-2e (defined as posing moderate hazards for carcinogenicity, genotoxicity, reproductive toxicity, developmental toxicity, and/or endocrine activity). Further distinction within BM-2e sub-benchmark scores may lead to greater differentiation of higher versus lower hazard BM-2s. Of the 25 BM-2e plasticizers, hazard classifications among 68% have low confidence in hazard classifications, based on modeling, limited or marginal evidence, or conflicting data for reproductive toxicity, developmental toxicity, or endocrine activity. The remaining 8 BM-2e plasticizers (32%) have reliable data supporting a moderate hazard classification for screened hazard endpoints. A higher confidence level in hazard classifications provides stronger support that these 8 plasticizers may pose a higher hazard than BM-2e plasticizers with low confidence levels. Examining trends in BM scores and sub-benchmark scores within a set of plasticizers provides insight into the identification of safer alternatives. Further research into filling data gaps and the use of robust chemical hazard frameworks to inform the design of safer plasticizer molecules can lead to a wider supply of lower-hazard plasticizer alternatives.

2234 A Survey and Quantitative Risk Assessment of PFAS Chemicals in Hair Care Products

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Per- and polyfluoroalkyl substances (PFAS) are a family of chemicals that are used in various industrial and consumer product applications. A recent analysis identified 13 different PFAS in approximately 200 personal care and cosmetic products. Limited information is available regarding potential exposures to PFAS following application of personal care and cosmetic products. Therefore, the purpose of this analysis was to 1) determine the concentration of 23 different PFAS in a survey of hair care products, 2) perform a quantitative risk assessment to calculate potential systemic PFOA exposures associated with these products, and 3) compare these values to potential systemic exposures from shower water containing PFOA. PFOA was selected as the comparison chemical for this quantitative assessment given the available toxicological data. Ten commercially available hair conditioner products were selected for analysis, and the PFAS content was assessed using modified EPA Method 537 Version 1.1. A daily systemic exposure dose (SED) to PFOA was estimated using (1) amount of product applied per application, (2) number of applications per day, (3) PFOA concentration in the product, (4) a retention factor, (5)

surface area of the scalp, (6) dermal absorption of PFOA, and (7) adult body weight. Low (mean amount applied and 2% dermal absorption) and high (95th percentile amount applied and 70% dermal absorption) models were evaluated to assess different parameters. A similar calculation was performed for shower water, accounting for number of showers per day and body surface area. No PFAS were detected in the analyzed products; therefore, we assumed that the hair conditioners contained half the PFOA method detection limit (2 ng/g). For the shower water assessment, we assumed that the water contained PFOA at the EPA health advisory standard (70 ng/g). Margin of Safety (MOS) values were calculated by comparing the SED results to the PFOA oral RfD (0.00002 mg/kg/day). The hair conditioner MOSs ranged from 17,900 to 795,000, while the shower water MOSs ranged from 4,230 to 434,000. Given the above assumptions, potential exposure to PFOA from hair conditioner use was approximately 50-75% lower than exposure to PFOA-containing shower water alone. These findings suggest that potential exposure to PFOA in the examined products is not associated with an increased health risk.

2235 Risk Assessment of Trihalomethanes in the Development of Public Health Goals for Drinking Water in California

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Disinfection is necessary for the elimination of microbial contaminants in drinking water. However, the disinfection process of chlorination or chloramination produces toxic chemicals, known as disinfection byproducts (DBPs). These DBPs include four thrihalomethanes (THMs): chloroform, bromoform, bromodichloromethane (BDCM), dibromochloromethane (DBCM) that are currently regulated federally and in California. The established federal and state maximum contaminant level (MCL) for total concentration of THMs in drinking water is 80 ug/L. The Office of Environmental Health Hazard Assessment (OEHHA) has proposed Public Health Goals (PHGs) that represent the concentration of each individual THM in drinking water that would not pose significant health risks to the public, including sensitive subpopulations (e.g., infants and children). PHGs are not regulatory standards and are based solely on public health considerations without regard to economic considerations. PHGs are used by the State Water Resources Control Board (SWRCB) to establish California's MCLs for drinking water. The proposed PHGs are: 0.4 ug/L for chloroform based on liver and kidney cancer; 0.5 ug/L for bromoform based on cancer of the large intestine; 0.06 ug/L for BDCM and 0.1 ug/L for DBCM based on liver cancer. OEHHA also developed health-protective concentrations for all four THMs based on noncancer effects.

2236 Risk Assessment of 1,1,1-Trifluoroethane (HFA-143a) as a Potential Impurity in HFA-134a in Metered Dose Inhaler Products

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Asthma prevalence in the United States has been steadily increasing in recent decades. According to a survey conducted by the Centers for Disease Control (CDC) in 2017, about 25 million people in the US, or 8% of the population, suffer from asthma. Treatments delivered by pressurized metered dose inhalers (pMDI) are the cornerstone of symptom management among asthma patients. The compound 1,1,1,2-tetrafluoroethane (HFA-134a) is a commonly used propellant in pMDIs. Here, we derived a permissible daily exposure (PDE) of 1,1,1-trifluoroethane (HFA-143a) as a potential impurity in HFA-134a and compared this to the current US Food and Drug Administration (US FDA) accepted specification for HFA-134a. Standard risk assessment methodology was followed, and the PDE was derived in accordance with available International Conference on Harmonisation (ICH) guidance. We determined that HFA-143a is not expected to be mutagenic or carcinogenic based on *in vivo* and *in vitro* mutagenicity and genotoxicity studies and a two-year chronic oral exposure study, in which the no-observed adverse effect level (NOAEL) was > 300 mg/kg bw/day, the only dose tested. No signs of developmental and reproductive toxicity were reported from inhalation exposure to HFA-143a in studies with rodents and rabbits. The most sensitive endpoint was determined to be cardiac sensitization hazard. In an acute inhalation study with beagle dogs, cardiac sensitization responses were observed with a no-observed adverse effect concentration (NOAEC) of 250,000 ppm, equivalent to a delivered dose NOAEL of 3,716 mg/kg. However, the two-year chronic study would provide a more appropriate point of departure for risk assessment given potential chronic pMDI use. Based on a NOAEL of 300 mg/kg determined from the two-year chronic oral exposure study, a PDE of 0.6 mg/kg was derived. We compared this PDE to the US FDA accepted impurity concentration of 20 ppm for HFA-143a in clinical-grade HFA-134a. Assuming an HFA-143a concentration of 20 ppm is delivered in one 50 µL pMDI actuation, a safety margin of 25,510 is derived. These results strongly suggest that the US FDA accepted concentration of 20 ppm for HFA-143a provides appropriate protection against potential adverse health effects caused by HFA-143a as a potential impurity in an HFA-134a pMDI.

2237 Analysis and Comparison of Major Immune Cell Populations in Peripheral Blood of Naïve Cynomolgus Monkeys

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Changes in major immune cell populations in the peripheral blood of cynomolgus monkeys, as determined by flow cytometry, are routinely used as an immunological endpoint in toxicology studies. However, it is difficult, especially for studies involving small animal groups, to determine a "normal" value that can be used as a guideline. To determine what constitutes a "normal" value for various immune cell populations in the peripheral blood, we analyzed large cohorts of naïve adolescent (total: n=168, males: n=84, females: n=84) and infant (n=33) cynomolgus monkeys. We determined relative percentage and absolute counts of immune cell populations, such as CD3+ total T cells, CD3+CD4+ T cells, CD3+CD8+ T cells, CD3-CD20+ B cells, CD3-CD16+ NK cells, and CD3-CD14+ monocytes. The number of CD3+ total T cells were 3.06±1.25, 3.24±1.35, 2.88±1.12, and 4.97±1.55 x 106 per mL for total adolescent, male, female, and infant cynomolgus monkeys, respectively. The number of B cells (CD3-CD20+) were 1.34±0.75, 1.45±0.90, 1.23±0.56, 1.85±1.01x 106 per mL and the number of NK cells (CD3-CD16+) were 7.59±4.24, 8.56±4.44, 6.63±3.81, 4.48±1.94 x 105 per mL for total adolescent, male, female, and infant cynomolgus monkeys, respectively. The numbers for monocyte counts (CD3-CD14+) were 2.48±1.09, 2.46±1.22, 2.50±0.94, and 4.44±2.08 x 105 per mL for total adolescent, male, female, and infant cynomolgus monkeys, respectively. Our results also indicated statistically significant differences in absolute counts of various cell populations based on the age and sex of cynomolgus monkeys. For example, infants had significantly higher levels of total lymphocytes, CD3-CD14+ monocytes, CD3+ total T cells, CD4+ T cells, and CD3-CD20+ B cells compared to adolescent monkeys ($p<0.005$, Mann Whitney test). The absolute counts of CD3-CD16+ NK cells, were significantly lower in infants compared to adolescent monkeys ($p<0.0001$, Mann Whitney test). In addition, we also observed sex-dependent differences where females had significantly lower counts of CD3+CD4+ T cells and CD3-CD16+ NK cells compared to males (($p<0.001$, Mann Whitney test). Overall, our data helps understand the various immune cell populations present in peripheral blood cynomolgus monkeys. The reported values can also serve as a guideline for improved understanding of the various toxicology related changes introduced by therapeutic treatments during drug development.

2238 Inter-Individual Variability Adjustment Factors for Occupational Exposure Limits

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Using a default adjustment factor of 10 for inter-individual variability (*i.e.*, pharmacokinetic (PK) and pharmacodynamic (PD) variability) is common practice in setting occupational exposure limits (OELs) to protect workers in the pharmaceutical manufacturing environment, though there is some evidence suggesting this value may be unnecessarily conservative. Active Pharmaceuticals Ingredients (APIs) are unique in that they typically have extensive human exposure data, including PK and PD data that can be used to calculate a chemical-specific adjustment factor (CSAF), which may be used in place of the default of 10 in calculating OELs. The purpose of this study was to evaluate the variability in internal exposures, primarily in healthy volunteers, to identify a reasonable default adjustment factor for most healthy workers. From the publicly available data on APIs, the means and standard deviations of exposure data, as area-under-the curve (AUC) after administration of the API, were collected. These data were used to calculate CSAFs for each API, as the mean AUC plus two standard deviations divided by the mean AUC. Using two standard deviations is conservative in that it covers >95% of the upper bound response in the population. The mean of the CSAF values for all the APIs evaluated rounded to 5, indicating that a default adjustment factor of 5 for an API would be representative of inter-individual variability within a subject population. Therefore, an adjustment factor of 5 for inter-individual variability would be appropriate for setting OELs that would protect most workers in pharmaceutical manufacturing. This default would not be recommended for compounds with certain sensitive subpopulations (*e.g.*, asthmatics or variant metabolizers) that are not part of the group assessed for calculation of the CSAF and might be included in the worker population.

2239 Setting Default Health-Based Exposure Limits for Early-Stage Monoclonal Antibody-Based Therapeutic Proteins

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Health-based exposure limits (HBELs) are developed for active pharmaceutical ingredients to minimize product cross-contamination in GMP manufacturing facilities (acceptable daily exposures, ADEs) and to identify acceptable exposures for worker protection (occupational exposure limits, OELs). In the absence of sufficient data to support a robust, compound-specific assessment and derivation (*e.g.*, early in the development of the drug candidate), default HBELs may be assigned. However, standardized methodologies for the establishment of default limits for biotechnology-derived products such as recombinant proteins and monoclonal antibodies (mAbs) have not been previously described. Robust data sets from 28 marketed mAb-based therapeutic agents were evaluated to derive ADE and OEL values utilizing the lowest therapeutic dose, standard adjustment factors, and adjustment for bioavailability. A comparison of these HBEL values identified three Hazard Categories based on therapeutic target. Recommendations were developed for assigning default ADE and OEL values of 0.1, 1, 10, 100 µg/day or µg/m^3 for early-stage mAb-based therapeutics, taking into account the assigned Hazard Category and potency. The criteria on mechanism of action, potency, and bioavailability should be reviewed by a qualified toxicologist, and the limitations of the assessment should be understood when assigning default values to ensure these are protective of the general population and/or workers, as appropriate. Limitations, including predictability of hazards for novel therapeutic targets and unpredictable adverse effects, should be understood. As the Hazard Category and the human dose may change as more information becomes available, there should be vigilance to ensure the defaults are updated and/or replaced with more robust, compound-specific derivations when sufficient data are generated.

2240 Rapid Evidence Mapping for Health: A Case Study

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Stakeholders in the field of environmental health are increasingly relying on tools and practices from the disciplines of evidence synthesis and systematic review to summarize the literature and identify scientific consensus with respect to potential health risks. Given the ever-accelerating pace of publications in this field, the practice of "evidence mapping" is being increasingly used to identify the key areas of study relevant to a given topic along with gaps in the literature. However, constructing detailed evidence maps can be resource-intensive, limiting their utility for practical implementation, particularly for broader questions of interest. As a result, approaches that increase the speed and reproducibility of evidence mapping are in great demand. Here we outline a process called "rapid Evidence Mapping" (rEM), which we define as a resource-efficient form of knowledge synthesis in which components of the systematic review process are simplified to produce a visual and quantitative representation of the scientific evidence. We show how rapid evidence maps can be created with the aid of Sciome's text-mining and machine learning software, and to illustrate the application of the procedure, we describe a proof-of-concept case study on the topic of low-calorie sweeteners (LCS) with respect to human dietary exposures and health outcomes. The resulting rEM produced similar findings compared to a traditional evidence mapping of the same topic (Wang et al. 2016), but required significantly less time, effort, and resources to create. Furthermore, a sensitivity analysis evaluating the set of studies included at 25% recall (i.e., the point at which the machine learning algorithms predicted we had identified 25% of all relevant references) would have resulted in the same conclusions regarding the current state of the science and existing research gaps. This observation suggests that further efficiency gains can be achieved by mapping only a computer-selected subset of the available literature. rEMs can be used to quickly summarize the available body of evidence relevant to a research question, identify gaps in the literature to inform future research, and contextualize the design of a systematic review within the broader scientific literature, significantly reducing human effort while yielding results comparable to those from traditional methods. The potential time savings of this approach make it a powerful tool for rapidly translating knowledge to inform science-based decision-making.

2241 Setting Permitted Daily Exposures (PDEs) for Pharmaceutical Agents Administered by the Intravitreal Route

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The eye is a complex and multilayered organ which possesses three chambers, the largest being the posterior chamber which houses the retina. This chamber is filed with vitreous humor (VH) which, although vascularized, functions independently of blood to eliminate foreign substances. Eyes possess a number of physiological barriers restricting or preventing movement of these substances, including therapeutic agents, between the eye and the general bloodstream. Because of the unique structure and functionality of the eye, existing guidelines for setting health-based Permitted Daily Exposures (PDEs) for pharmaceutical agents administered by the intravitreal (IVT) route have limited applicability. IVT drugs: 1) deliver a small volume of a highly concentrated dose, which may not be presumed to be systemically effective; 2) are formulated/engineered with the intent of causing little to no systemic toxicity; 3) may be formulated to locally target ocular tissues; or 4) may only migrate from the VH into the general bloodstream in very small amounts. Consideration must also be given to the pharmacokinetic behavior of IVT drugs once they have been injected into the VH, and to the actions of intraocular enzymes. For these and other reasons, calculation of an IVT PDE for a drug using direct, volume-based extrapolation from the eyeball to the human body or bloodstream may not be appropriate. Instead, three alternative approaches to setting IVT PDEs are proposed. Two may be described as "compliance- or quality-driven," while the third is dose-driven. The first approach may be applied to setting PDEs for small-molecule drug substances which are given exclusively by IVT injection, the second for small-molecule drug substances given both by IVT injection and systemically, and the third for high molecular-weight drugs, such as monoclonal antibodies and other biologicals. All three approaches are based on a review of readily available data for IVT-administered drugs, and all are considered scientifically supportable. The strengths and limitations of each approach are described. A comparison of the three approaches suggests that PDE values in the range of 1–20 µg/day are appropriate for drug substances present as potential contaminants in an IVT drug to which a patient might be exposed. IVT PDEs may also be used in manufacturing plants to set cleaning limits for shared equipment. This range applies only to pharmaceutical agents and not to other chemicals, for which further investigation is required.

2242 Assessment of the Applicability of Threshold of Toxicological Concern (TTC) for Per- and Polyfluoroalkyl Substances (PFAS)

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The presence of low amounts of PFAS have been reported in industrial and consumer products and thus there is potential for human exposure. Most PFAS have little or no toxicity information, thereby necessitating a resource-effective approach to screen and prioritize those that need further safety assessment. The TTC approach proposes a *de minimis* exposure value based on chemical structure and toxicological information from similar chemicals. Herein we test the applicability of the TTC approach to PFAS by incorporating a dataset of no-observable-adverse-effect-level (NOAEL) values for >20 PFAS chemicals into the corrected Munro TTC database. All substances were assigned into Cramer Class III (i.e., 1.5 µg/kg/day), and the cumulative distribution of the NOAELs evaluated. There was no statistical significance in the PFAS enriched distribution compared with the Munro distribution. The derived human exposure level for the PFAS enriched Cramer Class III dataset was >1.3 µg/kg/day. Evaluation of the structural chemical profiles showed that the added PFAS had distinct Toxprint Chemotypes. Dimension reduction techniques such as Principle Component analysis on the Toxprint Chemotypes showed clustering of the additional PFAS chemical away from the Munro TTC dataset. Similar calculations on whole chemical structures using maximum common substructures showed that the PFAS were distinct from the chemicals currently in the Munro dataset. However, the available data shows that PFAS NOAELs cluster within the substance in the Cramer Class III. Consideration of PFAS as a separate TTC class could result in a lower TTC value for these substances; however, at this time the relatively small data set inhibits any additional sub classification. This analysis shows that incorporation of these ~20 PFAS do not significantly change the current TTC Cramer Class III distribution and furthermore, provides an expansion of the chemical space providing support that the TTC approach can be applied to PFAS chemicals.

2243 Proposed Tier-Based Safety Evaluation for Personal Care and Cosmetic Products

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In the United States, the US Food and Drug Administration (US FDA) is responsible for the regulation of marketed cosmetic products. Currently, there are no laws or regulations that require specific safety testing, nor are companies required to share product safety information with the US FDA. In 2019, two senators introduced the Personal Care and Cosmetics Act, which would increase the regulatory oversight of the US FDA for personal care and cosmetics products; however, this bill contains limited information or proposed testing requirements. In the present evaluation, we propose a tier-based safety evaluation framework for personal care and cosmetic products. The proposed 3-tiered safety evaluation framework was utilized to assess the safety of a personal care product that has reports of adverse events following application. Specifically, Tier 1 involves an ingredient-based assessment to evaluate the safety of individual ingredients in the product and to identify any potential hazards associated with exposure. This includes methods for dermal hazard identification, supply chain analysis, and potential contaminant screening. Tier 2 involves a product mixture based assessment to evaluate the safety of the final formulated product. We present proposed methods for *in vitro* and in use testing for various health outcomes, as well as heat stress degradation of the product mixture following hair styling. Finally, Tier 3 is a population-based assessment that monitors the safety of a product post-market. This tier helps determine potential risk factors associated with reported adverse events and provides an example of a post-market product safety surveillance program. Overall, this testing strategy allows for efficient and focused testing, minimizes cost and time, and provides a comprehensive evaluation of a product and its ingredients.

2244 HGBEnviroScreen: Enabling Community Action through Data Integration in the Houston-Galveston-Brazoria Region

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The Houston-Galveston-Brazoria (HGB) region faces numerous environmental and public health challenges given the density of industrial facilities and activity associated with being the nation's energy capital. Historically disadvantaged communities most often impacted by natural disasters like hurricanes or industrial events such as chemical fires are limited by their ability to access and utilize big data for advocacy efforts. We developed HGBEnviroScreen, a geospatial tool that integrates data from multiple environmental and public health indicators, to identify and prioritize regions of heightened vulnerability in part to serve communities developing environmental justice action plans. This tool provides valuable information on critical factors driving vulnerability at the census tract level for an 8 county area. While similar in objectives to existing environmental justice tools such as EJScreen and CalEnviroScreen, HGBEnviroScreen is unique in its ability to integrate and visualize national and local data to addresses regional concerns, and serves as an innovative method that could be adapted or scaled for use by other metropolitan areas. For the 1090 census tracts in the HGB region, we accrued data into five domains: (i) social vulnerability; (ii) baseline health; (iii) pollutant exposures and risks; (iv) sources of pollution; and (v) flood risk. We then used the Toxicological Prioritization Index (ToxPi) methodology to integrate and visualize data across the indicators and domains. We found that the highest vulnerability census tracts generally had multifactorial risk factors, with the most common drivers being flood risk, social vulnerability, and proximity to pollution sources. As a result of combining ToxPi with broadly collected geospatial information, HGBEnviroScreen is not only helping inform HGB-area communities and decision-makers of the physical locations of greatest vulnerability, but is also providing insights into which domains would most benefit from improved planning, policy, and action in order to reduce vulnerability in the future. *This work was supported by a grant from NIH/NIEHS (P42 ES027704) and a contract from Environmental Defense Fund.*

2245 Tox21 Approaches for Reduced Animal Testing: Alternative Approaches to Hazard Identification and Dose-Response for Agrichemicals

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With the US Environmental Protection Agency (EPA) directive to reduce animal testing, including reducing mammal study requests by 30% by 2025 and eliminating them by 2035, alternative approaches to hazard identification, dose-response and exposure assessments, and risk characterization are critical. For chemical uses regulated through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), mammalian studies are critical to the hazard identification and dose-response evaluations of risk assessment. This project reviewed and evaluated points of departure (PODs) derived by regulatory agencies for conventional acute and chronic dietary and occupational risk assessments by pesticide type (insecticide, fungicide, or herbicide) and by chemical class. These PODs were compared with various crop-specific exposure estimates using tools such as commodity specific tolerance levels and dietary exposure models to determine margins of safety. The objectives of this project were to utilize the abundance of existing animal data to define POD ranges for use in risk assessment and to identify the key toxicity studies for various crop uses and chemical classes that are used to derive these PODs. This approach also supports the establishment of chemical class specific PODs as well as default worst case PODs for different use indications (insecticides for example). Utilization of existing data does allow prioritization of data generation such that only those studies critical for POD derivation will be conducted in future. Such an approach would significantly reduce and potentially replace the use of thousands of animals which is necessary to fulfill the data requirements providing a path forward to support the EPA's reduction of animal use directive.

2246 Streamlining Literature Capture: A Comparison of Automated Approaches to Identify and Categorize Literature for Review

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Systematic reviews that examine the risk of human exposure to environmental chemicals can be labor intensive, usually requiring human screening of thousands of publications for inclusion and categorization. Effective tools that facilitate grouping of literature to inform hazard identification, mechanisms of action, and toxicokinetics are needed to improve the planning and efficiency of systematic review efforts. We compared two approaches for collecting relevant mechanistic literature using the genotoxicity of chlorpyrifos as a test case: (1) using a set of specific keywords contained in CRAB 3, an online text mining tool that retrieves literature from PubMed using predefined but hidden keywords to identify and annotate cancer endpoint literature for user-designated chemicals; (2) using a broad set of keywords to retrieve a comprehensive set of literature from PubMed followed by machine learning to identify the relevant studies. For the latter approach, we used DoCTER, a text analytics tool that relies on supervised machine learning to identify and prioritize relevant studies, in two distinct ways: (i) using a previously annotated dataset to train the machine learning algorithm and (ii) using a customized active learning approach in which the DoCTER tool interacted with users during the algorithm training process and indicated when its predictive model had achieved the desired level of accuracy. DoCTER was also utilized to identify relevant studies from the CRAB-generated body of literature for inclusion. CRAB generated 177 records of which approximately 50% were determined to be relevant by expert review. In our second approach, DoCTER predicted 260 of 4,889 abstracts to be relevant. DoCTER predictions had a recall of 92% and a precision of 59% and resulted in a higher number of relevant studies retrieved than the CRAB approach. 96% of the studies identified by the CRAB approach were also identified in the second approach based on DoCTER. While CRAB is useful for initial scoping and mapping (especially for multiple chemicals and endpoints), it is limited by its use of hard-coded non-transparent keywords and high rate of capture of non-relevant literature. The PubMed-DoCTER approach resulted in a higher rate of capture of relevant documents but is less useful as a mapping tool. Thus, CRAB provides a rapid overview of literature categorization, while the PubMed-DoCTER approach is a more robust and transparent approach for in-depth assessments.

2247 Alice in Numberland: Mathematics of Variability and Uncertainty—Can Kinetics Help Us Understand Variability in Toxicity?

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In food safety assessment, risk characterisation requires the integration of external exposure (oral exposure), internal dose (kinetics), and toxicodynamic data. Here, a workflow allowing such integration is presented and is illustrated with examples from chemicals relevant for human health risk assessment. In 2015, EFSA funded collaborative research to characterise human variability in kinetics and toxicodynamics. Bayesian meta-analysis was used in order to derive pathway-specific uncertainty factors for several cytochrome P450 enzymes, such as CYP3A4, 2D6, 2C9, and 2C19, UGTs (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7) and transporters (P-gp, BCRP, OATs), which can replace the relevant subfactors within the overall default uncertainty factor of 100. In addition, the estimated variability of the enzymes can be used to allocate distributions of kinetic parameters derived from quantitative *in vitro-in vivo* extrapolations. Based on *in vitro* intrinsic clearance assays with primary human hepatocytes it was possible to determine isoform specific metabolism and kinetics parameters for chemicals. Human variability distributions for metabolic pathways were assigned to estimate lognormal distribution for the chemical clearance. These distributions were used in a physiologically based kinetic (PBK) model with Markov-Chain Monte Carlo. Human physiological parameters were derived from Popgen, and partition coefficients were estimated using quantitative structure-activity relationship (QSAR). The PBK model was applied to data-rich and data-poor case study chemicals, including chlorpyrifos, phosmet, triflumeron, and resveratrol. Moreover, variability in toxicodynamic data was assessed for the main metabolites of chlorpyrifos and phosmet, chlorpyrifos-oxon and phosmet-oxon, using human donor blood. Inhibition of acetylcholinesterase was measured for different donors and variability in toxicodynamics was incorporated in the PBK model, resulting in a PBK-TD model, incorporating variability in both kinetics and dynamics. The PBK(-TD) model is available on an open source modelling platform TK plate.

2248 A Comparison of Threshold of Toxicological Concern (TTC) Values to Reference Dose (RfD) Toxicity Values

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There are thousands of chemicals for which limited, or no hazard data are available to characterize human risk. For such chemicals, threshold of toxicological concern (TTC) values constitute a science-based tool that helps prioritize potentially hazardous chemicals for further evaluation. Herein we gauge the level of conservatism of TTC values by comparing them to more rigorously derived reference dose (RfD) values for 288 chemicals identified in the US Environmental Protection Agency's (US EPA) Integrated Risk Information System (IRIS) database. Using both the Cramer decision tree and the Kroes tiered decision tree approach to determine TTC values for these chemicals, ~80% and 86% of these chemicals, respectively were determined to be more conservative than their corresponding RfD values. The median log ratio of RfD/TCC values was ~0.74 and ~1.5 for the Cramer and Kroes approach, respectively indicating that RfD values were, on average, 5.5 to 32 -fold higher than their corresponding TTC values. An investigation into log ratios that were 2 standard deviations below the median (i.e. RfD<<TTC) identified chemicals that may need a second evaluation. Overall, this analysis indicates that TTC values appear to be health protective and are more conservative than RfD values. Also, RfD values that are significantly lower than TTC values for a specific chemical suggest the need for reevaluation.

2249 Interpreting Occupational Exposure Limits: An Analysis of NIOSH Recommended Exposure Limits by Health Endpoint

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Occupational exposure limits (OELs) are an essential tool for controlling worker exposures to toxic chemicals. OELs have been developed to protect workers from exposure to carcinogens, chemical irritants, sensitizers, neurotoxicants, and a variety of other toxicities. However, OELs are not all created equal. Many OELs are based on avoidance of chronic health effects, but others are based on acute health impacts. Health-based methods used to determine OEL values have ranged from a determined point of departure from an exposure-response relationship to quantitative risk assessment with extrapolation to a target risk level. In some cases, the OELs are not health-based, but are set based on the analytical limit of quantification or reliable quantitation limit. Still, the OEL (or a specified fraction of the OEL) is perceived as a "safe" level of exposure. In this presentation, thirty NIOSH Recommended Exposure Limits (RELs) are evaluated for the health endpoint basis of the REL, methods used for determining the OEL, target risk levels (where appropriate), and the analytical limit of quantification. The RELs are compared to the results that would be obtained from implementation of the NIOSH Occupational Exposure Banding eTool, and the EPA 8-hour time-weighted average (TWA) Acute Exposure Guideline Levels (AEGL). A description of the risk interpretations from the point of view of the risk assessor is compared with the point of view of the industrial hygienist. For example, the 8-hr TWA REL for ammonia is 25 ppm, with a STEL of 35 ppm. This is based on human observational studies showing respiratory irritation. Tier 1 occupational exposure banding would indicate that ammonia would be classified a band E chemical, corresponding to exposure concentrations of <0.1 ppm, and the AEGL-1 8-hour TWA value is 30 ppm (corresponding to acute health effects in the general population). Interpretation of the RELs, occupational exposure bands and AEGL values for ammonia and 29 other chemicals is discussed.

2250 Rapid Hazard Identification Using Human iPSC-Derived Cell-Based *In Vitro* Testing: A Case Study of a Hypothetical Spill of a Complex Substance

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Around a million tons of petroleum products are spilled worldwide annually as the result of accidents or negligent use. Assessment of the resulting human health and environmental hazards is challenging due to the chemical complexity of these substances. Crude oil consist of a multitude of naturally-occurring aliphatic and aromatic hydrocarbons, heteroatoms and other non-hydrocarbon molecules, while refined products may also contain proprietary additives. Here, we describe a case study involving the hypothetical spill of a real "oil blend" material whose composition is described only as "lubricant base oil (petroleum, various CAS numbers), >70%; and additives (proprietary), <30%". Due to this cryptic description of the product's composition, first responders and local authorities may require additional characterization of potential hazards in order to inform the choice of a cleanup standard. We hypothesized that *in vitro* bioactivity profiling in human iPSC-derived hepatocytes, neurons, and cardiomyocytes would be more informative than traditional chemical analytical data in performing rapid hazard identification. Our approach was to employ both traditional methods (EPA methods for trace metals, volatile and semi-volatile substances, as well as full-scan GC-MS) and bioactivity-based testing of the "oil blend" alongside two well-characterized petroleum refining products as well as a library of crude oils. Data were then presented to a group of professionals knowledgeable about oil spill response representing diverse stakeholders from industry and state agencies. Participants were asked to determine which type of information was most informative with respect to making decisions about the cleanup response of the "oil blend" in comparison to the other tested substances. The participants agreed that while rapid traditional chemical analysis was helpful to a limited degree, bioactivity testing data allowed for a more informed discussion of the potential hazards that may be present in the "oil blend," and provided a more confident basis for rapid decision-making in this hypothetical case study. *Funding for this research is by a grant from the National Academies of Sciences, Engineering, and Medicine's Gulf Research Program (GRP).*

2251 Deep Learning in Automated Text Classification: A Case Study Using Toxicological Abstracts

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Sponsor: S. Snow

Machine learning technology has been widely adopted as a cost-saving document prioritization approach in systematic literature reviews related to human health risk assessments. It is typically applied using supervised machine learning approaches that use a training dataset composed of a relatively small set of documents with human-annotated labels indicating the topic of each document to build models that automatically predict the labels of a much larger set of unlabeled documents. Deep learning algorithms form a branch of machine learning that relies on complex neural network architectures to learn the features of the object to be classified. Although deep learning algorithms have until recently mainly been applied for image, video, and audio classification, they are increasingly being deployed on text classification problems. To explore the potential advantages and practicalities of

using deep learning algorithms in the document prioritization step of systematic literature reviews, we compare the performance of the most commonly used deep learning architectures (MLP, CNN, bi-LSTM, CNN-bi-LSTM, and bi-LSTM-CNN) to a traditional machine learning model (SVM) using a dataset of approximately 7,000 toxicological abstracts. We find that SVM baseline performs well (F1 accuracy score range of 0.80 to 0.85), MLP performs marginally better (F1 accuracy score range of 0.82 to 0.88), CNN performs similarly well (F1 accuracy score range of 0.81 to 0.85), bi-LSTM performs worse (F1 accuracy score range of 0.75 to 0.86), CNN-bi-LSTM performs worse (F1 accuracy score range of 0.76 to 0.86), and bi-LSTM-CNN performs better than SVM (F1 accuracy score range of 0.83 to 0.88). The results indicate that deep learning does not offer increased performance over SVM in all instances. In addition, we found that deep learning requires considerably higher levels of algorithm training, run time, computational power, and algorithm tuning compared to the baseline traditional machine learning algorithm (SVM). For these reasons, we conclude that deep learning algorithms may not be the best solution for text classification problems.

2252 Evaluation of the Reproducibility and Gender Effects of a Five-Day *In Vivo* Screening Approach Using High-Throughput Transcriptomics to Estimate Benchmark Doses of Apical Outcomes

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Risk assessors use data from long-term toxicity studies to estimate regulatory exposure levels that pose minimal human risk. These studies are often prohibitively expensive and time-consuming. The DNTP is currently evaluating alternative methods to provide estimates of chemical exposure levels that induce minimal risk. Recently, high-throughput transcriptomics (HTT) has been proposed as a timely and cost-effective screening approach that covers a vast array of biological space. We previously evaluated the use of HTT in a short-term 5-day *in vivo* assay in male rats with the objective to determine if benchmark doses (BMDs) for transcriptional changes in the liver and kidney were similar with BMDs for traditional toxicological (apical) endpoints. For most of the 20 chemicals tested, the 'most sensitive' transcriptional pathway BMDs from the 5-day assays were within a factor of 5 compared to the 'most sensitive' apical (histopathological) BMDs estimated from historical NTP chronic or sub-chronic toxicity studies. Thus, the 5-day assay can be effectively used to provide reasonable estimates of BMDs for apical endpoints. In the current study, we evaluated the reproducibility and gender effects of the 5-day assay using 4 of the 20 chemicals. Male or female HSD rats were exposed for 5 consecutive days by oral gavage to 8 or 10 dose levels of TBBPA, BDCA, PFOA, or furan. Liver and kidney were collected 24 hr after the final exposure for transcriptomic analysis using the rat S1500$^+$ platform. Uterus was also collected from female rats. HTT dose-response data were analyzed using BMD Express 2.2 to determine transcriptional pathway BMDs for liver, kidney, and uterus. Preliminary data have shown that, although the identities of the 'most sensitive' transcriptional pathways active in liver and kidney differed across independent experiments (n = 3 in males and 1 in females), the pathway BMDs were similar. This suggests that the 5-day assay with HTT is reproducible with regards to estimating the BMDs, but not the identities, of the 'most sensitive' transcriptional pathways in liver and kidney.

2253 Evaluation of Model Selection Criteria Including Model Averaging during the Application of Benchmark Dose Method to Quantal Response Data

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To employ the benchmark dose (BMD) method in toxicological risk assessment, it is critical to understand how the BMD lower bound (BMDL) for reference dose calculation is selected following statistical fitting procedures of multiple mathematical models. The purpose of this study was to compare the performance of various model selection criteria, model exclusion criteria and model averaging methods for developing the guidance of BMD method for quantal response data. Simulation-based evaluation of model exclusion and selection processes was conducted, comparing validity, reliability and other model performances. Three different empirical datasets were analyzed for exposition, each yielding different characteristics of the dose-response pattern (i.e. datasets with rich information in high or low response rate, and approximately linear dose response pattern). The best performing criteria of model exclusion and selection was different across differently characterized datasets. Nevertheless, a model averaging method over three models with the lowest three AIC values (MA-3) did not reveal the worst performance, and MA-3 without model exclusion offered the best results among model averaging methods. Model exclusion including the use of Kolmogorov-Smirnov test did not necessarily improve the validity and reliability. The present study indicates that using MA-3 would be a recommended option whenever applicable. *Acknowledgement: This study was commissioned under a grant for the 2018 Cabinet Office Research for Assessment of the Effect of Food on Human Health, Japan (ID: 1801, PI: Akihiko Hirose).*

2254 Development of the Tool Kit for the Individual-Based Aggregate Exposure Assessment of Biocides Using the Multi-Use Pattern of Consumer Products

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Current exposure assessment methods for consumer product were focused on the consumer-only exposure level to diagnose the safety level of consumer product. There remains, however, another question: where or not current exposure distribution in the general population by consumer product is safe. To answer this question, different type of approach needs to be applied to assess the aggregated exposure in the individual level using the multi-use pattern of consumer product. Different from the individual food consumption rate for each food group, there is no exposure factor matrix for the individual aggregate exposure assessment of consumer product, which include the individual exposure intensity for each consumer product which they use. Until now, most exposure factors for consumer product were calculated from consumer-only survey for each consumer product. So we could not estimate the individual aggregate exposure from consumer products. In this study, we derived the multi-use pattern of consumer products for the artificial reference population from the separately conducted two survey data: (1) the consumer-only survey for exposure intensity factor(use frequency, use amount, and use time) of more than 200 people and (2) the individual utilization survey for every types of consumer products for 3,000 people. To estimate the exposure intensity factor for 3,000 people from consumer-only survey data, a probabilistic machine learning algorithm, Variational Inference Auto-encoder (VIA), was applied. Finally, in this study, a new toolkit as a chemical-independent modeling framework for the aggregate exposure assessment for multi-use consumer products was developed. The individual aggregate exposure assessment method was compared with traditional methods using the representative value (mean or 95th percentile values) of exposure factor and the random variables by Monte Carlo simulation. This toolkit was firstly applied to screen the more risky biocide compounds in consumer products and to refine the exposure reduction measures such as product safety standard for biocide compounds such as surfactants commonly used both for cleaning compound and a preservative in many kinds of liquid type of chemical products.

2255 Application of a Systematic Evidence Map to Characterize Factors That Modify Chemical Exposure or Response

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Systematic evidence maps use systematic review methods to identify the amount and type of evidence available to address a particular topic. They are used for identifying and categorizing chemical-specific literature that may be potentially relevant to human health risk assessment, and employ the use of systematic review screening approaches such as a Population, Exposure, Comparator, Outcome (PECO) statement and software applications such as Distiller SR. This study explores the use and feasibility of the systematic evidence map approach to provide a clearer picture of the available literature on modifying factors that may impact the relationship between chemical exposure and outcome. Using inorganic arsenic as an example, studies that were previously identified via a literature search as potentially having susceptibility information were screened and tagged at the title and abstract screening level using Distiller SR. Each study was screened by two reviewers. The modifying factors for susceptibility that were included in the analysis were alcohol consumption, co-exposure to other environmental contaminants, environmental justice, gender, genetic polymorphism, lifestages, microbiome, nutritional deficiencies, pre-existing disease, and smoking status. Results of this screening process were compared with search string results and as expected, two-person screening at the title and abstract level was more precise with the level of effort still being manageable. Genetic polymorphisms and nutritional

deficiencies are the most studied modifying factors. Some modifying factors, such as genetic polymorphism or smoking, were easier to discern than others, like environmental justice. The resulting evidence map gives a better understanding of available literature on each of the modifying factors with specific focus on the chemical of interest. This case study moves the application of evidence maps beyond the chemical exposure and outcome dynamic and allows for a deeper insight into the available literature especially for data-rich chemicals. *Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.*

2256 Safer and Less Environmentally Harmful Alternative Electrokinetic Approach to Extraction of Living Plants for Cosmetic Ingredients

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Plant extracts are widely used in cosmetics, mainly as bioactive ingredients. There is a growing trend to highlight the use of natural ingredients in cosmetic end use products to differentiate them in the marketplace. The increased interest in employing bioactive ingredients propels the demand for evaluation of differentiated plant extracts. Traditional extraction processes usually require high energy consumption and/or the use of large amount of solvents, which could result in unfavorable safety and environmental profiles. To address these concerns, we have used a new approach that preserves the inherent osmotic pressure of the plant cell juice and uses it as a separation medium with targeted fractions being mechanically and gently separated based on their electrokinetic potential without the use of solvents for isolating plant fractions for cosmetic use. The process starts with the separation of a stable intracellular colloidal dispersion from live plants. The dispersion is separated into different fractions via targeted destabilization. This process can eliminate or drastically reduce the presence of certain toxins (e.g., parthenolide, pheophorbides, patulin, 5-methoxypsoralen, and heavy metals). A cradle-to-grave lifecycle analysis shows that this process has a lower environmental footprint compared to traditional technologies in several respects. This includes reduced global warming potential, ozone depletion potential, acidification potential, photochemical ozone potential, eutrophication potential, primary energy demand, abiotic resource use, and land use. The E-factor (ratio of mass of waste per mass of product) is very low (≤1) for fractions made via this process, and almost zero (≅0) is achievable. For comparison, E-factors for bulk chemicals, fine chemicals, and pharmaceuticals typically are in the ranges of 1-5, 5-50, and 25-100, respectively. In summary, this alternative process: (1) enables more effective utilization of the underexplored potential existing in living plants, (2) allows targeting of multiple biological pathways with a single ingredient, (3) provides superior efficacy, safety and reproducibility, (4) utilizes a solvent-free process for fractionation, and (5) utilizes a process that has a lower environmental impact and generates less waste.

2257 Development of the Modeling Framework for the Cohort-Based Risk Assessment Approaches Based on the Longitudinal Individual Aggregate Exposure and Biomarker: Case Study for Dioxins

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New approaches for the risk assessment for the chemicals such as POPs with the long half-life in human body and has been developed because the traditional approach based on cross-sectional design have failed to assess the time trend of exposure level on the basis of the receptor. Cohort-based approach is an alternative for this type of chemicals, which can be applied to the life-long exposure assessment and the life-stage specific risk assessment. Critical parts in the part of exposure assessment of the cohort-based approach is the re-construcution of the past exposure and the projection of the future exposure, which can be derived by 'population kinetic model' depending on the emission trend and the half-life of the chemicals and confirmed by the longitudinal biomonitoring data. In this study, a systemic modeling framework was developed for the cohort-based risk assessment. The modeling framework is based on the individual level aggregate exposure assessment module including food consumption as a main exposure media for POPs such as PCBs and dioxins. Time trend of food concentration and individual food consumption rate on the basis of body weight and lipid content during the past 20 years were derived by nation-wide survey DB for about 10,000 Korean people every three years and applied to assess the individual external exposure distribution. The population kinetic model was used to predict the internal concentration of chemicals in the individuals with different body weight and lipid content along the ages from their cohort years from the external exposure amounts. Finally, the modeling framework will be used to compare the internal and external exposure in the individual level and applied to derive the kinetic parameters such as congener-specific half-life. The estimated kinetic parameter for each congener will be used to diagnose the current exposure levels and to project the future internal exposure level among Korean population. From this simulation can make it possible to assess retrospectively and prospectively the exposure distributions and to diagnose where or not current regulation measures is enough to reduce the exposure level below the safety guideline.

2258 Beauvericin and Zearalenone Metabolites Combinations Alter Viability and Oxidative Stress in Undifferentiated Neuroblastoma Cell Line

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Beauvericin (BEA) is an emergent mycotoxin produced by many species of *Fusarium* and by *Beauveria bassiana;* while Zearalenone (ZEA) by several *Fusarium spp.* Metabolization of ZEA originates two main derivatives α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL). BEA, α-ZOL and β-ZOL are present in stored grain and co-exposure through the diet is very common. Once ingested, it is possible to reach the brain and cause neuronal alterations. Combinations may cause increase of toxicity produced by these mycotoxins. In this *in vitro* study, it is presented the combined effects of all three mycotoxins in binary and triple mixtures on the viability and oxidative stress response of undifferentiated human neuroblastoma cell line (SH-SY5Y). Cell viability was assessed after 72 h using MTT assay and oxidative stress through reactive oxygen species (ROS) levels by malondyhaldehide (MDA) measurement during 120 min. Binary mixtures where α-ZOL was present revealed increase of ROS levels, while moderate levels where obtained in tertiary combinations. Our viability data showed that irrespective of the toxin combinations, the toxins have synergistic effect. BEA + β-ZOL and triple mixtures have induced a slight to high antagonistic response on ROS levels at low concentrations that have turned into strong synergism for high concentrations. This study clearly shows that co-contamination of food and feed with ZEA metabolites and BEA should be taken into consideration, as the co-exposure to mycotoxins might result in stronger adverse effect than resulted from the exposure to individual toxin. *Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R).*

2259 Effects of Nonnutritive Artificial Sweeteners on Lipid and Insulin Metabolism in the Model Organism *Caenorhabditis elegans*

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Obesity and type II diabetes (T2D) have reached epidemic proportions. Caloric reduction has been proposed as a primary intervention and treatment. The American Heart and Diabetes Associations have both endorsed the use of non-nutritive artificial sweeteners (nnAS) as a substitute for sugar. However, studies have suggested that the consumption of nnAS may disrupt both glucose and lipid metabolism. Based on this, we hypothesize that nnAS exposure would lead to increased lipid accumulation and disruption in insulin signaling. To this end we examined the effects of Sweet and Low® (SWL) or Splenda® exposure in the model organism *Caenorhabditis elegans.* Alteration in lipid accumulation was assayed in wild type N2 *C. elegans* with (SWL-10mM and 30mM), the active ingredient saccharin (SAC- 10mM) and glucose (GLU-10mM). Lipid deposition was assayed via the lipophilic dye Oil Red O' accumulation quantified through image analysis. Compared to control all SWL and SAC concentrations showed significantly more lipid accumulation, particularly around the digestive tract (p0.001). Insulin signaling disruption was evaluated using the TJ356 strain of *C. elegans*, which has a GFP tagged DAF-16 gene, a key component of the Insulin Like Signaling (ILS) pathway. DAF-16 (human ortholog FKHRL1) acts as a transcription factor that regulates the expression of genes related to fat metabolism, aging and stress resistance. Treatments consisted of control (CN), and 10mM or 30mM of Glucose (GLU), 10mM and 30mM of Splenda® (SP). Cellular localization of DAF-16 protein expression was evaluated using fluorescent microscopy imaging. Nuclear localization of DAF-16 expression was observed in all treatments when compared to control, indicating that DAF-16 is transported to the nucleus and therefore altering the regulation of fat, aging and stress response genes. SWL and SP exposure also increased lifespan when compared to glucose or control (27d CN, 25d SP, and 13d GLU), supporting the role of nnAS in ILS disruption. This was partially explained by the induction of dauer, a hibernation state, in SWL and

SAC worms which occurs in times of stress. These results indicate that nnAS are altering both glucose and lipid metabolism, although the mechanism and extent are still under investigation.

2260 Gap Analysis of Cannabidiol for Use as Novel Food in the European Union

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Cannabidiol (CBD) has been purported to aid in cognition and overall wellness; however, efforts to allow use of CBD in foods and dietary supplements has been met with resistance as many argue the science behind CBD has not caught up with these non-prescription uses. In early 2019, the European Commission decided that all extracts of hemp, and products including derived or synthetic CBD must undergo the Novel Food authorization procedure prior to being included in foods and dietary supplements. We have conducted an evaluation to determine if the existing data for CBD meets the criteria for use in Novel Foods as detailed in the European Food Safety Authority (EFSA) *Guidance on the preparation and presentation of an application for authorization of a novel food*. A common problem that arose was that many of the identified studies did not use purified CBD, and instead utilized hemp oil, CBD oil, or hemp extracts, all of which had different compositions and extraction processes. The studies evaluated included oral repeat-dose toxicity studies in at least 4 species (up to 3 months of dosing), reproductive and developmental toxicity (DART) studies, genetic toxicity studies, and sensitization. Three standardized genotoxicity assays using hemp extract were identified, in addition to 2 non-standard genotoxicity studies in human-derived cell lines. Additionally, while an *in vitro* (Ames) assay, *in vivo* (Comet and bone marrow micronucleus) assay, a 26-week toxicology study, and two DART studies were identified in the United States (US) Food and Drug Administration (FDA) Drug Review for Epidiolex (CBD), the full study reports are not publicly available and, thus, cannot be used to support a petition in the EU. As such, Tier 1 genotoxicity (bacterial reverse mutation assay [OECD TG 471] and *in vitro* mammalian cell micronucleus test [OECD TG 487]) and 90-day subchronic toxicology [OECD TG 408] studies with purified CBD should be conducted prior to submitting a novel food application. Depending on the results of the genotoxicity and 90-day studies, additional ADME studies, DART studies, chronic toxicity, and human studies may be required. Additional studies on the CBD metabolites would likely also be required due to observed interspecies differences in the metabolism of CBD.

2261 Modulation of the Pesticide Paraquat-Affected Molecular Pathways by Anthocyanins in *Caenorhabditis elegans*

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Anthocyanins are a group of C15 aromatic flavonoid compounds naturally existing in many food types, especially in fruits and vegetables. These phytochemicals have been shown to have multifaceted beneficial functions in the maintenance of human and animal health, with the most recognized being their free radical scavenging and antioxidant functions. However, we still lack a detailed understanding of how the metabolism of these compounds relates to their various functions. In this study, we conducted a metabolomic analysis to evaluate the modulation of metabolic profiling of anthocyanins in the nematode *Caenorhabditis elegans* treated with the pesticide paraquat (PQ) (0.1 mM to 0.9 mM). Briefly, N2 wide-type *C. elegans* were independently pretreated for 12 h with three anthocyanins (cyanidin, malvidin, and peonidin) at concentrations ranging from 10-100 μM, followed by treatment with PQ for another 12 h. After quenching with liquid nitrogen, a 3:3:2 v/v/v mixture of methanol/isopropanol/water was used to extract metabolites of worms at cryogenic temperatures. The extracts were freeze-dried via vacuum and then derivatized using the two-step methoximation/silylation protocol prior to analysis. The metabolites were detected by gas chromatography-time-of-fly/mass spectrum (GC-TOF/MS) and processed using multivariate statistical analysis. The metabolite sets were screened for further pathway analysis using rules of t-test (P) value < 0.05, and similarity value > 500. Our results suggested that PQ treatment primarily affected the metabolism of amino acids, energy, and lipids in *C. elegans*, and pretreatment with anthocyanins reversed PQ-impacted metabolic pathways. As compared to the metabolic profiling in PQ-treated and control worms, anthocyanins mainly protected two dominant metabolic pathways: amino acid metabolism and glycometabolism. Our findings demonstrate that metabolomic pathway analysis can provide a new perspective to better understand the protective effects of anthocyanins. Importantly, this could represent an effective method to elucidate the mechanism by which anthocyanins protect against chemical toxicant-induced adverse health effects.

2262 Quality Appraisal of Methodology for *In Vitro*, Nonclinical, and Clinical Studies Evaluating the Impact of Sucralose on the Gut Microbiota

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Sucralose is a non-nutritive sweetener approved by the US FDA for use in human food, with an acceptable daily intake (ADI) of 15 mg/kg body weight. However, there is recent debated evidence demonstrating that sucralose consumption may alter the composition of the gut microbiota. Therefore, in this study, a quality appraisal of methodology for published scientific literature was conducted to investigate whether the available data would support this claim. In total, 105 studies were identified; based on a defined set of inclusion criteria, 10 full articles and 6 conference abstracts were determined to be relevant (3 clinical, 10 non-clinical, and 3 *in vitro* studies). The design of each study (*e.g.,* dose, exposure route, duration, controls) and the reported outcomes related to the gut microbiome were critically evaluated. The 3 *in vitro* studies were determined to be of limited relevance to human exposure. Most of the non-clinical studies (9/10) were flagged with issues, such as exposures in excess of the ADI and/or lack of proper control groups, therefore limiting the significance of the reported changes on the gut microbiota. For the clinical studies, 2 conference abstracts reported that sucralose consumption can alter the gut microbiota; however, there was not enough detail in the abstracts to fully assess the studies. The 1 peer-reviewed full article reported that ingestion of sucralose (9.3 to 12.8 mg/kg body weight/day) for 7 days does not alter the gut microbiota in healthy adult male subjects. In conclusion, based on the quality of evidence, the available data do not provide a clear conclusion that sucralose modulates the gut microbiota at exposures relevant to human use in food.

2263 A Reassessment of Regulatory Reference Values and Background Exposure Levels for Heavy Metals in the Human Diet

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Heavy metals, such as arsenic, lead, cadmium, mercury, and chromium, are of concern in the food supply as oral exposures to these metals can cause adverse health effects such as organ damage, cancer, and neurodevelopmental effects. It is not possible to eliminate risks associated with exposure to heavy metals since they occur naturally in the environment and can also originate from anthropogenic activities that contaminate water, soil, and air. Therefore, it is important to periodically assess exposures from food and water and relate them to updated safe reference values on a national level. The objectives of this study were to 1) identify current regulatory reference values for heavy metals, 2) evaluate changes in reference values over time, 3) compare reference values set by different agencies, and 4) review total background level exposure from food and water to better understand the overall state of human exposure to heavy metals from consumption. Key scientific studies and endpoints used to derive current reference values were also compared across agencies. In general, heavy metal exposures to arsenic, cadmium, and mercury were below current regulatory reference values. Oral exposures to chromium were determined not to be a significant public health concern. However, total background level exposures to lead exceeded the new Interim Reference Level set by the FDA, especially for young children. Reference values and background-level exposure values presented here were also integrated into the International Life Sciences Institute (ILSI) North America Heavy Metal Screening Tool hosted by the Joint Institute for Food Safety and Applied Nutrition (JIFSAN). This tool allows users to utilize these values in an accessible website interface to conduct a screening-level risk assessment of heavy metal risks in foods and food ingredients. *This work was funded by the International Life Sciences Institute North America Summer Fellowship Program.*

2264 Mitigation of Mycotoxins in Fruit Juices by Pulsed Electric Fields (PEF)

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The population has increased the demand and consumption of foods with fresh-like. The emerging technologies, like pulsed electric fields (PEF) are effective at mild temperatures and use short treatment times, resulting in minimally processed food with less change of sensory and nutritional properties (1). Mycotoxigenic moulds may infect fruits during crop growth, harvest and/or storage. Mycotoxins produce toxic effects; aflatoxins (AFs) are

carcinogenic compounds and emerging mycotoxins [enniatins and beauvericin (ENNs and BEA)] are cytotoxic compounds (2). One industrial application of PEF is the treatment during fruits and vegetables processing. Two studies are available in bibliography about the reduction effect of PEF on AFs contents (3-4). The aim of the present work is to study the mitigation effect of PEF on AFB2, AFG2, ENNs and BEA contents in fruit juices containing seeds and vegetable roots. The samples were treated by PEF under field strength of 3 Kv /cm and specific energy of 500 KJ/kg. Then, the mycotoxins were extracted by dispersive liquid-liquid microextraction method (DLLME) and determined by HPLC-MS / MS-IT. After the PEF treatment, mycotoxins reductions around 40 and 60% were observed for ENNs and BEA and up to 70% for AFB2 and AFG2. Subsequently, degradation products formed during PEF treatment have been identified by HPLC-Q-TOF-MS. Acknowledgments: This research was supported by Conselleria de Educación, Investigación, Cultura y Deporte (AICO/2018/199) and the Ministry of Economy and Competitiveness (AGL2016-77610-R). *References: (1) Picart-Palmade, L., Cunault, C., Chevalier-Lucia, D., Belleville, M. P., & Marchesseau, S. (2018). Frontiers in nutrition, 5. (2) Marin, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Food and Chem. Toxicol., 60, 218-237. (3) Vijayalakshmi, S., Shanmugam, N., Ranganathan, K., Kumar, S., & Reddy, R. (2017). J. Food Process. Preserv., 41(6), e13230. (4) Vijayalakshmi, S., Nadanasabhapathi, S., Kumar, R., & Kumar, S. S. (2018). J. Food. Sci. Tech.,55(3), 868-878.*

2265 Subchronic Exposure to Cellulose Nanofibrils Modulates Gut Microbiome and Associated Metabolic Pathways

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It has been reported that cellulose nanofibrils (CNF) can decrease fat absorption and glucose release, suggesting their potential application as food additives or supplements in diets containing high contents of fat and sugars. However, the long-term effects of CNF uptake remained unknown. The purpose of this study was to determine the effects of subchronic CNF consumption at a physiologically relevant dose (30 mg/kg) by gavage on gut microbiome and associated metabolic pathways in Western diets (WD)-fed male C57BL/6 mice. The 16S rRNA sequencing analysis of fecal samples indicated that the gut microbiome communities were well separated on the unweighted Unifrac, an index of β diversity indicating presence/absence. However, after taking the difference in abundance into account, no clear patterns of separation on the weighted Unifrac among the groups were observed. Both the PD whole tree and chao1, indexes of α diversity that reflected the genetic diversity of the communities under study, were not significantly different among the groups. Further taxonomy profiling using LEfSe suggested that CNF treatment induced substantial shifts in gut microbiome when compared to either the vehicle or cellulose at the same dose. The *Clostridiaceae*, which was increased by CNF when compared to either the vehicle or cellulose, negatively correlated with the colon length and %body lean. The PICRUSt analysis of functional metagenomics identified multiple pathways (more than 50) that are either increased or decreased following CNF ingestion when compared to either the vehicle or cellulose treatment. Collectively, our study provides key findings regarding the safety of long-term CNF consumption associated with the ecology of gut microbiome and energy metabolism. *Supported by the USDA National Institute of Food and Agriculture [grant no. 2016-67021-24994/project accession no. 1009090], and in part by NIH [grant no. R41AT009523 and R41DK121553].*

2266 Absence of *In Vivo* Genotoxicity of a Purified *Aloe vera* Whole Leaf Juice Concentrate When Tested in Rats Using the Comet Assay

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Hydroxyanthracene derivatives (HADs) are a class of compounds naturally present in edible plants, including *Aloe vera*. HAD compounds such as aloin A, B and aloe-emodin found in the latex layer of *Aloe vera* leaf have been shown to be genotoxic in bacteria and mammalian cell assays, possibly attributing to colonic carcinogenicity observed in a 2-year rodent cancer bioassay of an orally administered *Aloe vera* whole leaf extract. In commerce, HADs in *Aloe vera* leaf are typically removed through an activated charcoal filtration process, also known as decolorization, during the production process of *Aloe vera* ingredients for use in foods and supplements. However, limited evidence is available regarding the *in vivo* genotoxicity potential of highly purified decolorized *Aloe vera* ingredients. The present study evaluated the *in vivo* genotoxicity potential of an orally administered purified *Aloe vera* whole leaf juice concentrate containing negligible levels of HADs (as test article [TA], < 0.3 ppm of aloins and non-detectable aloe emodin [LOQ = 0.02 ppm]). A GLP-compliant *in vivo* comet assay (OECD 489) was performed to test DNA damage in the colon and kidney following oral gavage administration of TA at 500, 1,000 and 2,000 mg/kg bw/day in male F344 rats for 2 consecutive days. Vehicle control (purified water), and ethyl methanesulfonate (EMS) were used as negative and positive controls, respectively. Rats administered TA exhibited no significant differences in final body weight compared to concurrent control animals. No other abnormal clinical observations associated with TA exposure were observed. For both kidney and colon, no dose level exhibited a statistically significant increase in DNA damage compared to the concurrent vehicle control group, and there was not a dose-related response. The positive control, EMS, resulted in statistically significant increases in DNA damage in the kidney and colon of the animals. The comet assay revealed that the purified *Aloe vera* whole leaf juice concentrate with *de minimis* HADs did not induce DNA damage in colon and kidney tissues under the experimental conditions described.

2267 Effects of Acrylamide on Isolated Rat Aorta and Mouse Phrenic-Nerve Diaphragm Toxicity

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Acrylamide (AA) can be formed during high-temperature frying or baking in the presence of both free asparagine and reducing sugars. AA could cause genotoxicity, carcinogenicity, neurotoxicity and reproductive toxicity. The mechanisms acrylamide induced vasotoxicity and neuromuscular toxicity are still unclear. This study is to investigate the possible mechanism of vasotoxicity and neuro-muscular toxicity of acrylamide. Vascular toxicity was studied by using an isolated rat aortic ring model, the aortic ring was divided into different doses group with or without endothelium, nitric oxide synthase (NOS) inhibition group (L-NAME), acetylcholine receptor (AChR) inhibition group (atropine and mecamylamine) and extracellular calcium inhibition. The changes in tension are to be an indicator of vasotoxicity. The nerve-muscle toxicity using a phrenic nerve-diaphragm model for testing, and experiments were performed AChR inhibition group and extracellular calcium inhibition group. The muscle stimulation changes are to be indicators of neuro-muscular toxicity and muscle contracture. The aortic ring results revealed that acrylamide caused relaxation in a dose-dependent when incubated with phenylephrine (PE) pre-induced endothelium-intact and -denuded aortic rings. Meanwhile, the half effective concentration (EC_{50}) values of acrylamide in endothelium-intact and -denuded aortic rings were 57.5 mM and 85.3 mM. The acrylamide-induced relaxation could be significantly attenuated by NOS inhibitor and AChR inhibitor (mecamylamine), and the other groups showed no significant inhibition. The phrenic nerve-diaphragm results revealed that acrylamide caused reducing stimulation and muscle contracture. There was no significant difference in the AChR inhibition group; the removal of extracellular calcium did not inhibit muscle contracture. These results indicate that vasodilatation induced by acrylamide is regulated by nitric oxide synthase via aortic endothelium. The regulation of muscle stimulation and contracture is caused by the nicotinic acetylcholine receptor.

2268 Multi-'omics Analysis to Understand the Mechanism Involved in Patulin-Induced Cell Transformation

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Patulin is a mycotoxin that naturally contaminates fruits and their products. Our unpublished results demonstrated that low dose chronic exposure of patulin leads to neoplastic changes in normal intestinal cells, thus, indicating the tumorigenic property of patulin. However, the mechanism(s) lying behind patulin's tumorigenic ability has not been fully understood. Therefore, in the current study, using a multi-omics approach, we identified the molecular and biochemical alterations accompanying patulin-induced tumorigenicity. Here, we performed RNA seq, high-resolution mass spectrometry and high-resolution magic angle spinning NMR technique for transcriptomics, proteomics and metabolomics. The differential analysis identified 163 transcripts, 297 proteins and 15 metabolites that were significantly altered in patulin transformed cells compared to passage-matched control cells. We observed that out of totals, 135 transcripts, 136 proteins, and all metabolites were significantly up-regulated, while 28 transcripts and 161 proteins were significantly down-regulated. Furthermore, analysis of gene expression data revealed that genes such as VCAN, SPP1, TNC, KCNN4, MUC5B, ETV4 have the potential to serve as prognosis biomarkers for the identification of early intestinal cancer patients. Moreover, the metabolomic study suggests that glutamine, taurine, leucine, valine, and alanine are the major altered metabolites. Among these, high levels of glutamine are very well reported in cancer cells. Similarly, leu-

cine, valine, and alanine found to be associated with cell proliferation and level of taurine could be used to predict the malignant transformation of certain tumors. In addition, serine and acetate act as a precursor for the synthesis of many other macromolecules. Furthermore, joint pathway analysis of proteomics and metabolomics indicated that (i) aminoacyl tRNA biosynthesis, (ii) glycosaminoglycan degradation, (iii) valine, leucine, isoleucine biosynthesis and (iv) glycosphingolipid biosynthesis pathways were deregulated in patulin transformed intestinal cells. Collectively, our findings provide insight into the biological reprogramming accompanying patulin-induced transformation in intestinal cells.

PS 2269 Boosting Levels of NAD+/NADH through NAD+ Precursor Promotes Mitochondrial Dysfunction

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Nicotinamide adenine dinucleotide (NAD^+) is both an essential co-factor and substrate derived from vitamin B3 and is intimately implicated in all essential bioenergetics. A decrease in intracellular NAD^+ levels is known to cause multiple metabolic complications and age-related disorders. Interest in pharmacological agents capable of increasing intracellular NAD^+ levels has driven attention to the use of dietary supplementation with NAD^+ precursors. One such NAD^+ precursor is dihydronicotinamide riboside (NRH), which increases NAD^+ levels more potently in both cultured cells and mice than current supplementation strategies with nicotinamide riboside (NR). However, the consequences of extreme boosts in NAD^+ levels are not fully understood. Here, we demonstrate the potential downside of acute NRH exposure in mammalian cells. Hepatocellular carcinoma (HepG3) cells treated with 100 μM of NRH showed slight but significant increase in cytotoxicity. NRH at 4 h exposure increased levels of oxidative stress by significantly increasing cellular reactive oxygen species (ROS). NRH substantially affected mitochondria health by altering mitochondrial membrane potential, increasing mitochondria superoxide formation and inducing mitochondrial DNA damage. As expected, NRH also causes metabolic dysfunction, altering mitochondrial respiration. Altogether, we demonstrated the adverse effects of acute NRH exposure in HepG3 cells. Extreme boosts in NAD^+/NADH likely have detrimental consequences in the liver cell model and further warrants to study these effects in other systemic models.

PS 2270 Estragole: DNA Adduct Formation in Primary Rat Hepatocytes and Genotoxic Potential in HepG2-CYP1A2 Cells

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Estragole is a natural constituent in herbs and spices and their essential oils. After CYP450-catalyzed hydroxylation and subsequent sulfation, estragole acts as a genotoxic hepatocarcinogen in rodents. Because of the genotoxic mode of action and the wide-spread occurrence in food and phytomedicines a refined risk assessment for estragole is needed. We analyzed the time- and concentration-dependent levels of the adducts N^2-(isoestragole-3′-yl)-2′-deoxyguanosine (E3′N^2dG) and N^6-(isoestragole-3′-yl)-desoxyadenosine (E3′N^6dA) in primary rat hepatocytes (pRH) in culture after incubation with estragole. We also investigated the genotoxicity in native and CYP1A2-overexpressing (HepG2-CYP1A2) HepG2 cells. pRH were incubated with estragole for different times. After cell harvest and lysis, DNA was extracted and enzymatically hydrolysed. The levels of the DNA adducts E3′N^2dG and E3′N^6dA were measured via HPLC-ESI-MS/MS using stable isotope dilution analysis. Genotoxicity was tested in the micronucleus assay incubating HepG2 cells or HepG2-CYP1A2 cells for 24 h with estragole und further 72 h without. Both adducts were formed in pRH and could be quantified after an incubation time of 1 h (E3′N^2dG: 1 μM; E3′N^6dA: 10 μM estragole). E3′N^2dG was the main adduct at all incubation times and all concentrations. E3′N^2dG could be detected after incubation at estragole concentrations as low as 0.1 μM after 24 h and 0.5 μM after 48 h. At all incubation times DNA adduct formation increased with increasing test concentrations, the concentration-response relationship showing indications for hypolinearity ('practical threshold'). DNA adduct levels were highest after an incubation time of 6 h and showed a downward trend at later time-points, possibly due to DNA repair. In the micronucleus assay no mutagenic potential of estragole was found in HepG2 whereas in HepG2-CYP1A2 cells 1 μM estragole led to a 3.2 fold and 300 μM to a 7.1 fold increase in micronuclei counts. In summary we could show, that DNA adduct formation from estragole is concentration- and time-dependent in pRH with E3′N^2dG as the main adduct. Both adducts could be quantified already after an incubation time of 1 h, E3′N^2dG being detectable already at 0.1 μM estragole after 24 h. The micronucleus assay confirmed the mutagenic potential of estragole.

PS 2271 Protective Effect of *Marinobacter hydrocarbonoclasticus* Culture Extracts against an Induced Oxidative Stress in Caco-2 Cells

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There is growing interest in the use of natural products to aid in the maintenance of human health. *Marinobacter hydrocarbonoclasticus* (strain CB08035) culture extracts CB08035-SCA (cultivated with yeast extract, soybean peptone and starch in artificial seawater) and CB08035-SYP (cultivated with casein and starch in artificial seawater) were found to show activity against *E. coli*, *E. faecalis,* and *P. aeruginosa*. Identified constituents by NMR spectrometry and MS from the extracts include phenylacetic acid, 4-hydoxyphenylacetic acid, 3-chloro-4-hydoxyphenyl acetic acid and a mixture of diketopiperazines. The study objective was to evaluate the cytotoxicity and the antioxidant response of Caco-2 cells to extracts CB08035-SCA and CB08035-SYP. Cytotoxicity induced by the extracts (10, 50, 100, 250 and 500 μg/mL), for 24 h incubation period, was assessed determining cell viability by lactate dehydrogenase (LDH) leakage and (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) MTT assays. Extract antioxidant activities by directly scavenging intracellular reactive oxygen species (ROS) and extract effects against a cellular oxidative stress induced by *tert*-butil hydroperoxide (*t*-BOOH) were also evaluated. Treatment of Caco-2 cells with extracts evoked no changes in cell viability, indicating that the concentrations selected did not damage cell integrity during the incubation period. Treatment of Caco-2 cells with extracts (100, 250, 500 and μg/mL) evoked a significant dose-dependent reduction in cellular ROS generation. The dramatic increase in ROS generation induced in Caco-2 cells by 200 μM *t*-BOOH was significantly reduced by half when the cells were pretreated with the extracts (250 and 500 μg/mL). The remarkable decrease in ROS levels and consequently in the oxidative stress condition leads to a decline of Caco-2 cell death. These findings support the use of these extracts for potential nutraceuticals purposes. *Work supported by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.*

PS 2272 Toxicological Risk Assessment of Pyrrolizidine Alkaloids: Investigations of the Hepatotoxic and Genotoxic Potential

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Pyrrolizidine alkaloids (PAs) probably represent one of the most significant groups of natural phytotoxins. To date, approximately 600 different PAs are known[1]. PAs can be found as contaminants in foods like teas, herbs and honey[2]. They are generally considered acutely and chronically hepatotoxic, genotoxic and carcinogenic[3]. For this reason, we generate data concerning *in vitro* cytotoxicity and genotoxicity of some food-relevant individual PAs. We want to provide data for a better risk assessment and further investigate the influence of PA structures on their toxicity as previously suggested by introducing interim Relative Potency Factors (iREPs)[4]. Genotoxicity of these selected PA congeners was determined in HepG2-CYP3A4 clone 9 cells[5] using the micronucleus test: monocrotaline, echimidine, europine, heliotrine, indicine, lasiocarpine, lycopsamine, retrorsine, riddelliine, senecionine and seneciphylline. Concentration-dependent increases in micronuclei counts were observed with most of the PAs. Cytotoxicity of PAs was tested in incubations with primary rat hepatocytes, HepG2 cells and HepG2-CYP3A4 clone 9 cells. They were tested at concentrations ranging from 1 to 300 μM. The cell viability was measured using the Alamar blue assay after 24 h and 48 h of incubation. In primary rat hepatocytes lasiocarpine (open-chained di-ester, 7S-structure) was the most cytotoxic congener, followed by the di-esters echimidine, retrorsine, riddelliine, seneciphylline and senecionine. The mono-esters heliotrine, indicine, europine and lycopsamine and the di-ester monocrotaline were much less cytotoxic. Similar cytotoxic effects were observed in HepG2-CYP3A4 clone 9 cells. In HepG2 cells, lacking CYP3A4, none of the selected PAs showed cytotoxicity in the concentration range tested. Genotoxic potencies of the congeners in the micronucleus assay were in good agreement with the cytotoxicity data although some PA-specific differ-

ences were found. *References: [1] CONTAM (2011) EFSA Journal 9(11):2406; [2] Allemang et al. (2018) Food Chem. Toxicol. 121, 72-81; [3] Fu (2017) Chem. Res. Toxicol. 30, 81-93; [4] Merz and Schrenk (2016) Toxicol. Lett. 263, 44-57; [5] Herzog et al. (2015) J. Cell. Biotech. 1, 15-26.*

2273 Effect of Magnesium and Silicon Oxides Nanoparticles on Immunological Alterations Induced by Mycotoxins in Rats

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Mycotoxins specially Aflatoxin B1 (AFB1) is considered one of the major hazardous secondary metabolites. AFB1 is well-known as a feed borne-hepatotoxic and immunosuppressive mycotoxin. This study was conducted to evaluate the efficacy of nanocomposite magnesium oxide and silicon oxide (MgO-SiO2) in reducing the toxic effects of AFB1 on the immunity of adult male Sprague Dawley rats. Animals were divided into a control (Gp1) and three experimental groups (Gps); Gp2 received feed contained 200 ppb AFB1, Gp3 received feed contained 200 ppb AFB1 and 0.5 g/kg MgO-SiO2 nanocomposite. While, rats of Gp4 received feed contained 0.5 g/kg MgO-SiO2 nanocomposite. Cellular and humoral immune responses, as well as caspase-3 expression in liver, spleen, and intestine, were all evaluated. Residual concentration of AFB1 was determined in serum, liver and fecal samples. The obtained data were statistically analyzed. AFB1 markedly reduced body weight gain and food and water consumption. Cellular immune response (total and differential leukocytes count, neutrophils phagocytic activity, lymphocyte transformation, macrophage activity and serum lysozyme activity), serum total protein, and humoral immune response (fractions of protein as estimated by SDS- PAGE electrophoresis) were all severely reduced by AFB1. These findings suggested that the nanocomposite MgO-SiO2 has high affinity to adsorb AFB1 and can effectively modulate its toxicity in rats. Therefore, Nanocomposite MgO-SiO2 may offer a novel effective and cheap approach for the preventive management of aflatoxicosis in animal.

2274 Are Dried Soybean (*Glycine max*) Seeds a Dietary Source of Metals (Al, Cd, Pb, Ni)?

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The soybean (*Glycine max*) is a legume with high nutritional value with a world production of 320 million tons. Many soy-based products (soy milk, soya cheese, soy sauce, fermented derivatives (tofu, miso), protein powder, lecithins, oil, dried beans, sprouts) are commercialized. Some nutritional aspects of dried soybeans have been studied but the toxicological evaluation of its metal content has not been assessed. Metals such as Al, Cd, Pb and Ni are toxicologically relevant as there are fixed TWI (tolerable weekly intake): 1 mg Al kg bw/week, 2.5 µg Cd/kg bw/week, 4.41 µg Pb/kg bw/week (nephrotoxicity), 19.6 µg Ni/kg bw/week. Objectives (i) to determine the content of Al, Cd, Pb and Ni in dried soybean seeds marketed for direct dietary consumption; (ii) to evaluate the exposure to Al, Cd, Pb and Ni derived from the consumption of soybeans in different consumption scenarios (25 g/day and 50 g/day). The content of Al, Cd, Pb and Ni has been determined in 39 samples of soybeans using ICP OES (inductively coupled plasma - optical emission spectrometry). For the estimation of intakes and their contributions to TWI, a body weight of 81.1 kg in men and 66.9 kg in women and various consumption scenarios have been used. Considering the mean concentrations detected, Al (6.31±2.6 mg/kg), Cd (0.03±0.01 mg/kg), Pb (0.06±0.01 mg/kg) and Ni (1.88±1.4 mg/kg), a consumption daily of 25 g/day would mean intakes of 158 µg Al/day (% TWI: 1.36% men, 1.65% women), 0.75 µg Cd/day (% TWI: 2.60% men, 3.14% women), 1.5 µg Pb day (% TWI: 2.94% men, 3.56% women) and 47 µg Ni/day (% TWI: 20.6% men, 25.1% women). The consumption of 50 g/day of soybean seeds would mean an intake of 316 µg Al/day (% TWI: 2.73% men, 3.31% women), 1.5 µg Cd/day (% TWI: 5.20% men, 6.30% women), 3.0 µg Pb/day (% TWI: 5.90% men, 7.12% women) and 94 µg Ni/day (% TWI: 41.2% men, 50.2% women). Men would reach 100% of the TWI of Ni with a consumption of 121 g/day and women would reach 100% of the TWI of Ni with a consumption of 99.6 g/day. Soybean seeds are a dietary source of metals, especially Ni. Soybased products should be considered as an additional food group in studies estimating total dietary intakes of metals and other nutritional compounds. Monitoring the contamination of soybeans with toxic metals should be implemented and limits for these metals in all soybean's products should be established.

2275 High-Voltage Atmospheric Cold Plasma (HVACP) Efficacy to Degrade Aflatoxin B1, B2, G1, and G2 and Safety of Aflatoxin Degradation Products

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Mycotoxins, particularly aflatoxins (AFs), are of public health importance because they cause a number of negative human and animal health impacts. They are secondary metabolites produced by fungi, i.e., *Aspergillus flavus* and *A. parasiticus* and are four different types of AFs, B1, B2, G1, and G2. Aflatoxins are very toxic to humans and animals; they cause liver cancer, associated with immunosuppression, stunting, and at high doses will lead to death. Human exposure to aflatoxins is the result of ingestion of contaminated foods, such as cereal grains or milk (M1 toxin) produced by animals previously exposed to aflatoxins in feeds. Cold plasma is a physical, novel non-thermal method, which is currently used in food industry. Cold plasma can refer to "gas-derived mix of atoms in their quasi-neutral ionized forms mainly composed of photons, ions, and free electrons as well as atoms in their fundamental or excited state with a net neutral charge". The plasma is generated using only atmospheric air and electricity. In this study, plasma was generated using High Voltage Atmospheric Cold Plasma (HVACP) operated at 85 kV and 60 Hz and generated 180 W. Pure aflatoxin (B1,B2, G1, and G2) was dissolved into chloroform, and an equivalent of 200 µM poured onto watch glasses until complete dry. Watch glasses containing aflatoxin were placed into boxes (27.31 cm x 17.78 cm) with 4.44 cm of gap distance, sealed into hermetic plastic bag, and directly treated in duplicate for 2, 5, 10, and 20 min with HVACP system described above. After the treatment, boxes were left over night to allow generated reactive species recovering their fundamental state into air. Aflatoxin residuals were extracted with chloroform and analyzed with LC-MS/MS. Preliminary results showed that aflatoxin B1 and G1 ("1") were sensitive to HVACP system; the aflatoxin B1 showed a reduction 90% after 2 min of HVACP treatment and aflatoxin G1 74% decrease after 2 min. After 10 min, the aflatoxin G1 residuals were below the limit of detection. Aflatoxin B2 and G2 ("2") were reduced for 38% and 79%, respectively after 20 min HVACP treatment. The large variability seen with B2 and G2 reduction likely results from the primary difference between the "1" and "2" toxins, which is the presence of a double bond at C8-C9 position, and readily degraded by reactive gas species. This double bond is absence in B2 and G2. The reduction of this C8-C9 double bond significantly lowers the aflatoxin toxicity. Chemical analyses are on-going to confirm these hypotheses. Future studies will correlate the aflatoxin degradation to toxicity. Toxicity assessment will be performed using *in vitro* methods using HepG2 cell and test for cytotoxicity, DNA fragmentation, and apoptosis.

2276 Analysis of Gallic Acid in *Hyperacanthus amoenus* and *Carissa bispinosa* Foliages

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Sponsor: M. Davis

Overproduction and accumulation of reactive oxygen species (ROS) and free radicals in living organisms are associated with adverse physiological effects such as ageing, cancer, cardiovascular and inflammatory diseases. Naturally occurring polyphenols including gallic acid (GA), have emerged as strong anti-oxidants and radical scavengers, and are thus potentially beneficial to organisms. The abundance of GA in feed has anti-nutritive and toxic effects. It affects bioavailability of micronutrients by forming insoluble complexes, induces apoptosis and interferes with signalling pathways. Hence, the objective of this study was to carry out aqueous extraction of commercial feeds, *Hyperacanthus amoenus* (HA) and *Carissa bispinosa* (CB) foliages. The extracts were subjected to quantitative analysis of GA levels as putative anti-nutritive and toxic factors using HPLC-DAD and HPLC-VWD. The quantification method was validated for linearity, accuracy, precision (repeatability, reproducibility, intra- and inter-day variability), limits of detection (LOD) and quantitation (LOQ). Furthermore, GA recoveries (n=6) in feed were 111 (% RSD=1.36), 94 (% RSD=1.04) and 92% (% RSD=2.10) at 2 ppm, 4 ppm and 6 ppm fortification levels, respectively, whereas in CB they were 103 (% RSD=2.42), 97 (% RSD=1.71) and 90 % (% RSD=2.38), and in HA, 114 (% RSD=1.51), 91 (% RSD=1.76) and 107% (% RSD=2.51). The determined LOQ in feed, CB and HA were 0.25, 2.39 and 0.032 ppm, respectively, whereas LOD were 0.083, 0.79 and 0.011 ppm, respectively. Repeatability (n=9) of CB and HA resulted in 1.17 and 2.33 % RSD, respectively, and reproducibility (n=9) of 1.72 and 0.46 %RSD. The GA contents of 2.00±0.04, 10.9±1.22 and 14.1±1.36 mg/g DW were obtained in feeds, CB and HA, respectively. These results were significantly different ($p<0.05$). These methods indicate that GA contents in selected plants may have adverse health and growth performance effects on livestock and can provide a valuable basis for quality determination of GA in plants.

2277 Evaluation of Immunological Effects of Dietary E171, a Food Grade TiO_2

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E171, food grade titanium dioxide (TiO_2), is a common additive in many foods. A recent study reported that exposure of rats to E171 in drinking water, after pretreatment with 1,2-dimethylhydrazine (DMH) (a gastrointestinal carcinogen and inflammagen) induced inflammation in the gastrointestinal tract, increased the percentage of dendritic cells (DC), and decreased $T_{regulatory}$ (T_{reg}) cells in Peyer's patches. Here, rats received food *ad libitum* containing increasing doses of E171 (0, 40, 400, or 5,000 ppm) for a total of 100 days and were evaluated for tissue specific and systemic changes in immune parameters. Food consumption was similar over the 100-day period between groups with maximum total exposure to E171 being 29,400 mg/kg in 100 days. No significant differences were observed in any E171 dose groups in the percentage of DC, $CD4^+$ T or T_{reg} cells within Peyer's patches or the periphery, compared to control diet fed animals. No E171 associated changes were observed in the profile of cytokine production in plasma, sections of jejunum, and colon in E171 alone fed rats. Significant differences were observed for IL-17A in colon (400 ppm E171+DMH) and IL-12p70 plasma (40 ppm E171+DMH). Thus, there is minimal to no effect of E171 administration in diet for 100-days on the immune system. *This work was supported by Michigan State University Center for Research on Ingredient Safety, the Grocery Manufacturers Association, the Titanium Dioxide Manufacturers Association, the International Association of Color Manufacturers, and the Fred and Pamela Buffett Cancer Center Tissue Sciences Facility Shared Resource, supported by the National Cancer Institute under award number P30 CA036727.*

2278 Differential Tissue, Cellular, and Molecular Sensitivity between Metabolic Syndrome Models Induced by Fructose and High-Fat Diets

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Metabolic syndrome (MetS) is a complex metabolic disorder with multidimensional etiology that encompasses diverse symptoms such as hyperlipidemia, abdominal obesity, insulin resistance, and hypertension. Both high fat diet (HFD) and high fructose diet have been associated with the increased prevalence of MetS worldwide and research at the bulk tissue level have shown involvement of different tissues in its pathogenesis. However, cell-specific mechanistic research has been limited by the previous lack of high throughput single cell techniques. Thus, the paucity of systemic investigation across different tissues and cell types has prevented a holistic understanding of MetS pathogenesis. Here we used single cell RNA sequencing to examine thousands of individual cells from hypothalamus and peripheral metabolic tissues (liver, adipose, and small intestine) from both HFD- and fructose-induced MetS models and chow-diet fed control mice. t-Distributed Stochastic Neighbor Embedding (tSNE) analysis showed altered cell expression profile in different dietary exposure and tissues. To further quantify these differences, we applied Euclidean distance-based measurement to quantify transcriptome change under dietary exposure. Differential cellular sensitivity was found in both dietary exposure models, such as stronger alterations in the transcriptome of hypothalamic neurons in fructose-induced MetS and more prominent changes in adipose cell types in HFD-induced MetS. We further used co-expression network modeling to characterize cell-cell interactions across tissues and revealed altered cellular communication circuits in each MetS model. For example, the tight interactions between hypothalamic cell types and adipose mesenchymal stem cells appeared to be weakened in MetS models, and a stronger immune component was observed in high HFD induced MetS. Finally, humanin (*mt-Rnr2*), a mitochondrial gene linked with metabolic homeostasis, was found to be differentially regulated between the two MetS models. Our results indicate that HFD and fructose diets promote MetS by engaging different cell types, modulating cell type specific molecular pathways, and rewiring within-cell and between-cell gene networks across endocrine and metabolic tissues. The identification of major cell types and regulators of different subtypes of diet-induced MetS will facilitate precision medicine for MetS.

2279 Inactivation of Encysted Muscle Larvae of *Trichinella spiralis* in Pigs after Mebendazole Drug Treatment

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The production of safe and healthy food products represents one of the main objectives of the food industry. The presence of microorganisms in meat and products containing meat can result in a range of human health problems, as well as economic losses to producers of these products. However, contaminated meat products continue to initiate serious and large-scale outbreaks of disease in consumers. *Trichinella spiralis (T. spiralis),* which is the cause of trichinellosis (also known as trichinosis), is a parasitic nematode found in many warm-blooded carnivores and omnivores, including pigs. *Trichinella spiralis* has a long-standing association with pork products, not only in the US but around the world. Infections occur worldwide, but are most prevalent in regions where pork or wild game is consumed raw or undercooked. Therefore, it is necessary to evaluate new therapeutic and/or chemoprophylactic strategies to treat or prevent infection in animals maintained in management systems that do not preclude infection. We evaluated the effect of 4 anthelmintic treatments on the viability of *T. spiralis* encysted muscle larvae (ML) in pigs. Pigs were infected at 8 weeks of age with *T. spiralis* (ISS 44) first stage muscle larvae (ML) obtained by artificial digestion of infected rat muscle. At 55 days post-infection (PI), pigs were randomly assigned into 5 groups: control (sterile saline), levamisole, mebendazole, doramectin, or moxidectin. Pigs were humanely euthanized on day 66 PI. Muscle larvae were then isolated from pig tissues by artificial digestion, collected, and counted to determine worm burdens in muscles of treated pigs versus control animals. These ML were then inoculated into mice for 30 days isolated larvae mice to assess viability. Mice were then euthanized and ML were then isolated from mouse tissues by artificial digestion, collected, and counted to determine worm burdens in muscles of treated mice versus control animals. Results indicated that only mebendazole treatment had toxic effects on *T. spiralis* ML in pigs and ML viability in mice. In conclusion, this experiment provides a way to eliminate *T. spiralis* and its potential to cause illness from infected pork.

2280 Application of Machine Learning on Food Safety Data Analysis

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With the development of new technologies such as next-generation sequencing (NGS) in pathogen detection and surveillance, the size of data in clinical and public health laboratory is increasing dramatically, including meta data. The major challenges currently exist on the interpretation and analysis of large size of data. In previous studies, we have developed a framework to pursue data mining on NGS datasets by topic modeling, which is an active research field in machine learning and has been mainly used as an analytical tool to structure large textual corpora for data mining. In this study, we continued the research on applying machine learning algorithms on the functional genomic analysis of NGS data. An NGS data set of 323 *Salmonella* isolates was retrieved from National Center for Biotechnology Information (NCBI) database, and SNPs were generated using our previously developed framework. Random Forest (RF) and Supporting Vector Machine (SVM) algorithms were then applied on both the SNPs dataset the SNPs corpus to predict the serotypes of *Salmonella*. High prediction accuracy and specificity were obtained in both datasets, with even better performance in the SNPs corpus. The implementation of topic modeling and other machine learning algorithms provides a new way in food safety data analysis. The machine learning algorithms can be applied on various datasets of different sizes to elucidate genetic information and potential biomarker identification, which is especially useful in big data era.

2281 The Analytical Method for the Determination of Polycyclic Aromatic Hydrocarbons from Milk Product Samples in the South Korea Market

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Polycyclic aromatic hydrocarbons (PAHs) are substances composed of two or more aromatic rings. The International Agency for Research on Cancer (IARC) determined that benzo[a]anthracene (BaA), chrysene (CRY), and benzo[b]fluoranthene (BbF) are possibly carcinogenic to humans (group 2B), and benzo[a]pyrene (BaP) is carcinogenic to humans (group 1). Additionally, the

European Food Safety Authority (EFSA) suggested that PAH8 (BaA, CRY, BaP, BbF, benzo[k]fluoranthene (BkF), indeno[1,2,3-c,d]pyrene (IcdP), dibenzo[a,h] anthracene (DahA), and benzo[g,h,i]perylene (BghiP)) are suitable indicator for carcinogenic PAHs in foods. In our study, we validated PAH8 using the method of ultrasonication and gas chromatography with mass spectrometry (GC-MS) in milk. Benzo[a]pyrene-d12 and chrysene-d12 were used as internal standards. In the present study, we obtained 35 milk product samples from national supermarket chains in 10 cities with a population of more than 1 million people in South Korea. In order to represent the analyzed products, we composited samples in one. The results indicate that the recovery of PAH8 from milk product samples ranged from 80% to 110%, precisions (RSD, %) were within 20%, and the all of R^2 was above 0.99. The limit of detection and limit of quantification of PAH8 were ranged from 0.037 to 0.097 μg/kg and 0.114 to 0.295 μg/kg, respectively. The result revealed that in 35 milk product samples, B(a)P and PAH8 content were significantly higher in stir-fried butter (0.210 μg/kg and 1.780μg/kg, respectively) and PAH4 (BaA, CRY, BaP, BbF) contents were significantly higher in infant formula milk powder (0.637 μg/kg).

2282 Total Arsenic and Arsenic Speciation Concentration of Rice-Based Food in South Korea

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Arsenic is a type of toxic metalloid occurring in the environment that can be absorbed into various organisms, especially rice plants. Rice is the major route of arsenic intake through food and was usually consumed as steamed rice. However, nowadays, there are many processed foods made of rice such as cake and cracker. The average daily consumption of rice in South Korea is up to 200 g per day, including the intake of rice-based food. In 2016, South Korea's Ministry of Food and Drug Safety (MFDS) established the regulation on the concentration of inorganic arsenic in rice less than 200 ng/g. Nevertheless, there is a lack of research on the concentration of arsenic species in rice-based food. In this study, total arsenic (tAs) and four arsenic species (arsenite (As (III)), arsenate (As (V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA)) were analyzed in steamed rice (n = 22), rice bread (n = 14), rice cracker (n = 15), rice wafer (n = 4), rice liquor (n = 16), and rice drink (n = 7) samples. All of the samples were purchased in 2019. For the present study, inductively coupled plasma-mass spectrometry (ICP-MS) was used to analyze tAs and high-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC/ICP-MS) was used to analyze 4 species of arsenic. The result revealed that no As was detected in rice liquor and rice drink. Also, tAs concentration in all samples was under 200 ng/g, and inorganic arsenic concentration in all samples was under 100 ng/g. Such findings have shown that in South Korean markets rice-based food has low risk issues based on the level for inorganic rice arsenic standard from the MFDS.

2283 Total and Arsenic Speciation Analysis in Rice-Based Products for Infants in South Korea

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Rice-based products can be a major source of arsenic since rice easily absorb arsenic from soil and water compared with other grains. Rice is widely used for infant food in which infants are more sensitive and have a low resistance to the toxicity of heavy metals. The toxicity level of arsenic depends on chemical form and inorganic arsenic (iAs) is more toxic than organic arsenic. The objective of this study was to investigate the total arsenic (tAs) and four arsenic species (arsenite (As (III)), arsenate (As (V)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA)) in dry form of baby food (n = 9), paste form of baby food (n = 15), rice snack (n = 13), rice drink (n = 6) and others (n = 5) samples. For the present study, tAs was analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) and separation of 4 species of arsenic was analyzed by high-performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). In our study, the predominant arsenic species were As (III) and DMA. In paste form of baby food and rice drink, As (V) or MMA were not detected. Total and inorganic arsenic content was found to have a similar tendency except others. Total and inorganic As content was found to be the highest in dry form of baby food, followed by rice snack, rice drink, paste form of baby food. The result of this study can provide a basis data for assessing the risk of exposure to As from ingestion of rice-based products.

2284 Analytical Method Validation for Polycyclic Aromatic Hydrocarbons in Edible Oil Using GC-MS and Content Change by Cooking Methods

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Polycyclic aromatic hydrocarbons (PAHs) in edible oil are known to be affected by the environmental contamination, manufacturing process or the nature of oil. The content of PAHs in edible oils is affected by cooking methods. We selected eight edible oils including soybean oil, rice bran oil, grape seed oil, rapeseed oil, sunflower seed oil, olive oil, corn oil, and perilla oil that are consumed with high frequency in the South Korean markets. The oil samples obtained from the representative stores distributed in South Korea were pooled, homogenized, and cooked by five methods including raw, stir-frying, pan-frying, boiling, grilling, and deep-frying. In this study, a liquid-liquid extraction method was applied to determine 8 different compounds of PAHs (PAH8) (benzo[a]pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylen, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene). Gas chromatography with mass spectrometry (GC-MS) was used for qualification and quantification. The accuracy varied between 90 and 110%, and the precision was within 20%. The linearity (r^2) was greater than 0.999. The limit of detection was 0.084 μg/kg, and the limit of quantification was 0.255 μg/kg. The values of PAH8 decreased by 0.2 to 1.8 μg/kg with boiling, while increased by 0.1 to 0.5 μg/kg with deep-frying. Also, stir-fried perilla oil had the highest value of PAH8 (5.5 μg/kg).

2285 Prenatal Nutrition Measured by *My Nutrition Index* Is Associated with Birth Weight and Cognitive Function in Children at Seven Years

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Nutrition is a multi-faceted, complex construct, where good nutrition should be associated with improved health outcomes. This is particularly true during pregnancy when prenatal maternal nutrition may impact the child's development. Most research papers on nutrition focus on individual nutrients and health outcomes. In contrast, our focus is on a holistic measure of nutrition. *My Nutrition Index* (MNI) is an index that measures the nutrient quality (i.e., "nutritiousness") of a specified daily diet and is calculated based on quantification of dozens of macro- and micronutrients that are specific to an individual's nutritional needs (as defined by published recommended guidelines for individual nutrient target ranges) by incorporating dietary restrictions, subject characteristics, activity level, and health behaviors. Other nutrition indices are based on scored food groups consumed and may not adequately adjust for micronutrient inadequacies during pregnancy. The Swedish Environmental, Longitudinal, Mother and child, Asthma and allergy (SELMA) study is a pregnancy cohort in Värmland, Sweden, with prenatal endocrine disrupting chemicals (EDC) exposure and dietary data available, making it possible to test for the potential mitigating effect of good nutrition on health effects from EDCs. Using prenatal nutrients from food frequency questionnaire (FFQ) data to construct an individual's MNI, the index is significantly and positively associated with important metabolic (as measured by birth weight) and cognitive function at age 7 years (as measured by IQ-WISC) in children when adjusted for co-variates. Regression models included both prenatal concentrations of an EDC (bisphenol F, PFOA) and MNI demonstrating the adverse association with EDCs and the positive association of a nutritious diet during pregnancy. Thus, MNI is evidently a metric of the general nutritiousness of daily diets and is useful in environmental health studies in representing the impact of good nutrition. *We gratefully acknowledge support from NIEHS: #R01ES028811.*

2286 Investigation of the Effect of Cold Plasma on *Aspergillus flavus* and *Aspergillus parasiticus* Inoculated on Raw and Processed Pistachio During Storage

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Pistachio is one of the most important horticultural products in the Iran which is very important in export and economy of Iran. One of the serious issues that endanger the export of this crop today is aflatoxin poisoning in pistachio production. Aflatoxin is a secondary metabolite of molds such as *Aspergillus fla-*

vus and *A. parasiticus* that poses many risks to human health. Plasma technology is one of the non-thermal methods to reduce the microbial burden and toxins produced on the surface of fresh and processed foods. In this study, 84 pistachios (42 crude and 42 processed) were obtained from Kerman province. And pistachio levels were disinfected by 0.3% sodium hypochlorite and the initial aflatoxins of the samples were measured by HPLC. *Aspergillus flavus* and *Aspergillus parasiticus* which were lyophilized and were prepared from Tehran University of Veterinary Medicine It was cultured in culture medium (SDA) and incubated at 25 ° C for 7 days. Then mold spores were removed from the plate was sprayed on pistachios and kept in 25 degree incubation for days 3, 7, 5, 10 and 21. The samples were divided into two groups and were exposed to plasma radiation for 10 minutes on the third and fifth days, on the seventh day for 15 minutes and on the 21st day for 20 minutes and at 30 KV. Then chemical tests including determination of fatty acid profile and peroxide number as well as determination of aflatoxins (B1, B2, G1, G2) were done three times in completely randomized block design using SPSS16 software. The results showed that plasma had no significant effect on chemical parameters (plasma penetration power is low) but it had a significant effect on pistachio aflatoxins and plasma radiation of helium origin had a more effective effect than plasma radiation of oxygen- origin. Therefore, it can be concluded that cold plasma can be used as a non-thermal method to reduce pistachio toxins.

PS 2287 A 90d Subchronic Toxicity Evaluation of Blueberry Polyphenols in Ovariectomized Sprague Dawley Rats

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Fruit and vegetable derived polyphenols are associated with numerous health benefits when consumed regularly. Because of this, consumers are increasingly turning to dietary supplements, which contain concentrated extracts of polyphenol-rich fruits and vegetables, to increase their consumption of these compounds. However, the safety of this consumption modality has not been adequately vetted. To address this, the safety of purified blueberry polyphenols was investigated over 90d in female, ovariectomized (OVX) Sprague-Dawley rats, following OECD 408 guidelines. Animals were dosed via oral gavage at 0, 50, 250, or 1000 mg total polyphenols/kg bw/d, and monitored daily. No differences in behavior, body weight, or food consumption were observed. Upon study completion, a complete necropsy was performed, with tissues harvested for histopathology and blood and urine collected for hematology, serum biochemistry, and urinalysis. No tumors or other macroscopic changes were observed at necropsy. Histopathological analysis did not show differences among treatment groups. A few statistically significant differences were observed in blood (total cholesterol and chloride ion concentration) and urine (color, pH, and bacterial counts) analyses, though these parameters were within normal ranges for the animals and not considered biologically significant. Intestinal permeability was tested using the FITC-dextran method. Increased gut permeability was observed in the high dose group, though no morphological differences were observed histopathologically in any section of the GI tract. Given the lack of changes noted elsewhere, the importance of the difference in intestinal permeability is likely of minimal physiological importance. These results indicate that the NOAEL for blueberry polyphenols in OVX-SD rats is ≥1000 mg total phenolics/kg bw/d. This translates to consuming ~10 g polyphenols in a 70 kg human, which is higher than the amounts present in commercially available dietary supplements.

PS 2288 Evaluation of Drug Interactions Using Primary Turkey Hepatocyte Cultures

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Accurate prediction of drug clearance in food-producing animals is critical for human food safety through prevention of violative drug residues. Although common in the commercial turkey industry, concurrent use of two or more drugs in the same feed can result in drug-drug interactions (DDIs) that may impact drug metabolism and clearance. Primary hepatocyte cultures have been used successfully for the development of models to assess drug metabolism in various species, however, data on hepatocyte cultures in poultry are extremely limited. The objective of this study was to establish and validate primary turkey hepatocyte monolayer cultures for use as a high-throughput *in vitro* model to screen for DDIs between drugs used in combination in poultry feed. Hepatocytes were isolated from the livers of adult male turkeys under general anesthesia using collagenase perfusion followed by culture in monolayer. The functional activity of cultured hepatocytes was assessed by monitoring albumin concentrations in culture media by ELISA and by evaluating gene expression of cytochrome P450 (CYP450) enzymes predominantly involved in poultry drug metabolism. Albumin levels peaked on the third day of a five-day period, with a maximum concentration of 232.72 ng/ml. RT-qPCR analysis of CYP1A4/5, CYP2C23, CYP2C45, and CYP3A37 revealed that CYP3A37 is the most abundant isoform expressed. The inducibility of the key CYP450 enzymes was assessed following treatment with known CYP450 inducers: rifampicin, phenobarbital, or 3-methocholanthrene. Treatment with phenobarbital resulted in a 50-fold and a 15-fold increase in CYP2C23 and CYP2C45, respectively. Drug depletion experiments have begun, with hepatocyte cultures being treated with either fenbendazole (anthelmintic), monensin (ionophore antibiotic), or fenbendazole+monensin at an initial concentration of 1µM for each drug, in quadruplicate. Tissue culture media samples were collected at 0, 0.5, 1, 2, 4, and 24 hours and analyzed by validated mass spectrometry. Linear regression analysis will be used to evaluate differences in the depletion rate for the drugs alone versus the drugs in combination, suggesting a DDI. Preliminary results of the first drug combination experiment show substantial depletion of both drugs within 24 hours. In order to achieve adequate statistical power, the experiment will be repeated in more birds. Additional combinations of drugs will be tested as part of the project.

PS 2289 Assessing the Effect of Gastrointestinal Digestion on the Pro-Inflammatory Potential of Dietary Advanced Glycation End Products Using an *In Vitro* Model of the Gastrointestinal Tract (TIM-1)

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Protein- and sugar-rich food products processed at high temperatures contain large amounts of dietary advanced glycation endproducts (dAGEs). Our earlier studies have shown that specifically protein-bound dAGEs induce a pro-inflammatory reaction in human macrophage-like cells. To what extent these protein-bound dAGEs survive the human gastrointestinal (GI) tract is still unclear. In this study we analysed gastric and small intestinal (SI) digestion of dAGEs using a validated, standardised *in vitro* digestion model: the TNO gastroIntestinal Model (TIM-1) is a dynamic *in vitro* model that is able to mimic much of the human GI tract. This model takes multiple parameters into account, such as: dynamic pH curves, peristaltic mixing, addition of pancreatic digestive enzymes, and passive absorption. Samples of different digested food products were collected after (i) only gastric digestion and (ii) after both gastric plus SI digestion at different time points. Samples were analysed using UPLC-MS/MS for Nε-carboxymethyllysine (CML), methylglyoxal-derived hydroimidazolone-1 (MG-H1), and glyoxal-derived hydroimidazolone-1 (G-H1). All AGEs were quantified in their protein-bound and free form. After gastric digestion, only protein-bound AGEs were detected and no free AGEs. After 2 hours of SI digestion, approximately 10% of the protein bound AGEs were hydrolysed, the other fraction still remained bound to proteins. The results of this *in vitro* study give a strong indication that protein-bound dAGEs survive gastrointestinal digestion. This also indicates that dAGEs enter the human GI tract with potential pro-inflammatory characteristics. These findings might have severe health implications for the general population and even more for susceptible patient groups. These results are part of a larger study on the health risk of dAGEs in processed food.

PS 2290 Burden of Disease of Neurodevelopment Impairment Associated with Cassava Cyanide in the Democratic Republic of the Congo (DRC)

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Cassava is an important dietary staple in many tropical nations worldwide, mostly in sub-Saharan Africa. It contains naturally occurring cyanogenic glycosides that can cause both acute and chronic diseases in humans. It is especially risky in child populations suffering conditions of famine and conflict, as cassava is more likely to be consumed without proper processing to remove the cyanogenic glycosides. The World Health Organization (WHO) recently estimated the global burden of diseases caused by cassava cyanide associated with konzo, an irreversible spastic paraparesis of sudden onset. But recent studies have shown that exposures to cassava cyanide can also impair neurocognitive health among human children who do not develop konzo. Additionally, children with konzo not only suffer from the paralysis, but also from neurocognitive and motor deficits. This will increase the over-

all burden of disease of cassava cyanide. The aim of the present study is to calculate a more comprehensive estimate of burden of disease caused by cassava cyanide exposure in the Democratic Republic of the Congo (DRC). We describe the concentration-response relationship for cyanide in cassava flour and children's Intelligence Quotient (IQ), the occurrence of cyanide from cassava flour, the integration of concentration--response and exposure into estimates of incidence of intellectual disability (ID), and the translation of incidence estimates into DALYs. The average IQ decrements was 0.38 attributable to dietary exposure of cyanide among children in DRC. The median incidence of mild (65<IQ<69), moderate (60<IQ<64), severe (50<IQ<59), and profound (IQ<50) ID was 5.43×10^4, 2.48×10^4, 1.78×10^4, and 2.15×10^3 respectively. On the basis of the potential years of life lost due to intellectual disability and life expectancies, the estimated DALYs were calculated as the cases in each ID category and the total cases using age 2 as the time of onset for the disease, which resulted in the loss of DALYs at 1.37×10^6 in DRC. We recommend educational efforts of interventions are recommended to reduce the presence of cyanide in the diet of at-risk populations in DRC and other sub-Saharan African countries.

2291 Surveillance of Aflatoxin Contamination Levels in Household Maize for Human Consumption in Kenya, East Africa

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Aflatoxins [AFs] are a group of naturally occurring mycotoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus* and other related *Aspergillus* fungi. Staple food items including maize and maize products, peanut, sorghum, tree nuts, and spices are highly susceptible to AFs contamination. There are four groups of AFs in order of toxicity namely AFB_1>AFG_1>AFB_2>AFG_2. AFB_1 is a group I human carcinogen, an immunosuppressant, an anti-nutritional agent, and most recently, have been implicated to contribute to growth impairments in children. In this study, we determine the occurrence and level of AFs contamination in freshly harvested maize for human consumption in rural Kenya to establish the current database for exposure assessment. Up to 338 maize kernels and freshly milled flour samples were collected from households in Siaya and Makueni counties of Kenya. Samples were analysed for AFB_1, AFB_2, AFG_1 and AFG_2 using Ultra High-Pressure Liquid Chromatography with Fluorescence detection. AFs were detected in 100% of the samples with the range of 2.14 - 411 µg/kg. The geometric mean of total AFs in all samples from Makueni County is 62.5µg/kg with 95% CI: 53.7, 71.4 while in Siaya County is 52.8 µg/kg with 95% CI: 44.0, 61.7. More than 90% of samples exceeded the maximum limit of 10 µg/kg. Makueni County is a hotspot for AFs contamination with numerous reported outbreaks of aflatoxicosis which is poisoning and deaths attributed to consumption of AF contaminated maize. Before this study, little to no surveillance data was available for AFs contamination in food supplies produced and consumed in Siaya County. This study showed that AFs contamination is prevalent in maize based foods in both counties and efforts should be undertaken to prevent adverse health outcomes in human populations.

2292 Safety Assessment for 5 mg/day Zinc in Dietary Supplement Products without Co-Supplementation of Copper in the 50+ Populations

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The Tolerable Upper Intake Levels (UL) for zinc, established by authoritative or scientific review groups, are designed to ensure absence of adverse effects on relevant indicators of copper status, with the value of 25 mg/day set by EFSA being the most conservative UL. The current assessment aimed to model the impact of 5 mg/day (low dose) supplemental zinc (without supplemental copper) on the zinc/copper ratio in older adults, aged 50 +. An analysis of the most recent National Health and Nutrition Examination Survey (NHANES) data (2013-2014) found that the typical ratio of zinc/copper intake, defined as the median was 8.6 in 50 + men (5^{th} to 95^{th} percentile ranging from 4.83 to 18.88) and 7.9 in 50 + women (5^{th} to 95^{th} percentile ranging from 4.25 to 16.78). Modelling of the data using 5 mg/day supplemental zinc (w/o copper) showed that the median ratio increased to 13.2 and 13.5 in 50 + men and women respectively, bringing 26.2 % of the males and 22.6 % of the females into a more favorable range (8.5 : 1 - 12.5 : 1) of zinc/copper intake ratio from nutritional perspective. Meanwhile, 16 % population of 50 + men and 29 % population of 50 + women had their zinc/copper intake ratio increased beyond the typical ranges of zinc/copper ratio (>95^{th} percentile) coming from diet only. However, these individuals have a relatively low baseline dietary intake for both copper and zinc. The exposure to the total zinc (even after 5 mg/day zinc) in the majority of these individuals remained below the most conservative EFSA UL of 25 mg/day for Zinc, meaning that such exposures are deemed to not adversely impact copper status. It is further interesting to note that zinc deficiency is more detrimental to copper status when copper intakes are low than when they are sufficient, thus the impact of zinc supplementation in these individuals may arguably be of greater nutritional benefit. In conclusion, for the US adults aged 50+ years, a low dose of supplemental zinc (w/o copper) does not adversely impact on human health and the overall level of risk is assessed as low when considering the established safe levels of zinc and the benefit this supplementation may bring.

2293 Can the Effects of Green Tea on Gut Microbiota Be Sustained?

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Studies have shown that green tea polyphenols can influence gut microbiota associated with a decrease of obesity and diabetes in mice fed a high-fat diet. This present study aimed to determine whether the effects of green tea polyphenols on gut microbiota (after 5 weeks of treatment of mice fed a low-fat diet) can be sustained after termination of the treatment in mice challenged with a high-fat diet. C57BL/6J (6-week old) male mice were fed a low-fat diet for 5 weeks. All 4 experimental groups, with 8 or 7 mice per group, were given drinking fluid containing 0.1% citric acid to stabilize the polyphenols and mask the bitter taste of tea preparations. During the 5 week period, one treatment group was given 0.6% green tea extract (GTE) and the other 0.2% epigallocatechin-3-gallate (EGCG) in drinking water. This time period is critical as most of the gut microbiome changes occur. Body weight, and diet and water/treatment consumptions were measured 3 times a week. The body weights for all groups increased steadily. The diet and water consumptions for all groups were approximately constant throughout the 5 week period. However, liquid consumption in the EGCG group was lower than other groups. After 5 weeks of treatment, all groups were switched to a high-fat diet (except one control group) with drinking water (no tea preparation) for 2 weeks. Body weight increased significantly for all 4 groups. The water consumption increased greatly at week 6, and then slightly declined by week 7. Diet consumption slightly decreased and remained constant throughout two week period. Fresh fecal samples were collected at week 0, 5, 6, and 7. Fasting blood glucose levels were measured at week 6 and 7 after treatment was removed. The control group with HFD was higher compared to other groups, but was not significant. In conclusion, no sustained effects (reduction in body weight and blood glucose levels) were observed due to the treatment.

2294 Characterizing the *In Vitro* Immunomodulatory Effects of *Echinacea purpurea* Products as a Strategy for Test Article Selection

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Echinacea is continually one of the best-selling supplements in the mainstream market for use as an immune stimulant to lessen the symptoms of the common cold or infections. *Echinacea purpurea* (EP) is one of the most popular species used by consumers even though there is very little safety data for use in children, pregnant women, immune-compromised individuals, or those taking EP products while on other medications. Similar to other botanical products, EP is a complex mixture with little known about its bioactive constituents, and additional concerns regarding product quality (i.e. contamination or adulteration). The National Toxicology Program (NTP) procured a variety of EP products to screen for immune effects *in vitro* and assist in the selection of a representative test article for future *in vivo* toxicity testing. Twenty-nine EP products (bulk material or finished), 6 reference materials, and 6 *Echinacea*-specific constituents were evaluated for immunomodulatory activity using a whole blood assay system. Cells were cultured in the presence of EP with and without stimulation with LPS or anti-CD3 antibody and anti-CD28 antibody and the secretion of proinflammatory cytokines (IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNF-α) was measured using a multiplex immunoassay. EP preparations were also evaluated for endotoxin levels to address potential impact on cytokine secretion. Endotoxin contamination was detected in 16 EP samples, 3 samples could not be evaluated due to assay interference, and 10 samples had no detectable endotoxin (<5 EU/mL). Three of the 10 endotoxin-free samples (EP-22 > EP-35 > EP-13) led to strong increases in constitutive cytokine levels. However, cytokine levels after

EP costimulation with LPS or α-CD3/CD28 were increased the most by treatment with EP-35. Additionally, EP-22 suppressed LPS-stimulated production of several cytokines. These results were integrated with phytochemical profiles of each sample (generated by nontargeted chemical analysis) to better describe the relationship between biological activity and chemical profile of EP products. Combining biological and chemical data, EP-35 was selected as the NTP test article for further *in vivo* testing.

2295 Pharmacological Evaluation of *Brugmansia sauveolens* Leaf Extracts and Its Fractions for Anti-Asthmatic Properties

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Asthma is one of the most common chronic diseases in developing countries with high air pollution especially in India. It causes respiratory problems with moderate to severe episodes. In present study, we evaluated one of the common herbs, currently available in local Himalayan region, *Brugmansia suaveolens*, also known as 'White Angel trumpet' for its anti-asthmatic properties. The leaf extract and fractions of the leaves were prepared and phytoconstituents, alkaloids, flavonoids, steroids and phenolic were characterized using TLC ad HPTLC. In preliminary *in vivo* study on Guinea pigs showed significant increase in cough latency time, decrease in cough frequency and increased antioxidant level. Further mechanistic evaluation for efficacy for receptor, beta-2, M3 (for bronchodilator) and H1 (for hypersensitivity) using molecular docking study was done. *In vitro* testing for involvement of leukotrienes (LTs), interleukins (ILs), transforming growth factor (TGF)-B, and prostaglandins (PGs) inhibition were also performed using human lung epithelial cells. The efficacy and toxicity testing displayed similar protective effect on the lung cells. These results suggest that the phytoconstituents of *Brugmansia sauveolens* have a potential to be used therapeutically for treatment of asthma.

2296 Nuclear Receptors and Transcriptional Regulation of Drug-Nutrient Interactions

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Drug-nutrient interactions are defined as physical, chemical or physiologic relationships between a drug and a nutrient. This association can result in altered absorption, distribution, metabolism, function, catabolism and excretion of either the drug or the nutrient upon co-administration or -exposure. Drug metabolism enzymes (DMEs) are transcriptionally controlled by xenobiotic-sensing nuclear receptors and transcription factors including pregnane-X-receptor (PXR), constitutive androstane receptor (CAR2 and 3), farnesoid-x-receptor (FXR), vitamin-D-receptor (VDR), arylhydrocarbon receptor (AhR) and Nuclear factor erythroid 2-related factor 2 (NRF2). In addition to many drugs and man-made chemicals, these proteins have naturally-occurring bioactive molecules, such as plant-based polyphenols, that act are modulators of their activity. Thus, nuclear and xenobiotic receptors are important mediators of drug-nutrient interaction by affecting the expression and hence activity of DMEs. We developed a work-flow to examine the potential of botanical extracts to cause drug-nutrient interaction through modulation of DME transcription, in a rapid and efficient manner. Botanical extracts are first examined in a mixture of optimized cell-based reporter assays, called the Drug-Nutrient Interaction (DNI) panel, consisting of assays for PXR, CAR2, CAR3, FXR, VDR, AhR and Nrf2. Any extract that results in a significant increase in reporter gene activity in the DNI panel is subsequently examined for relative contribution of each member of the panel to determine mechanism of action. The alteration of transcript levels for DMEs are examined upon treatment of the human hepatocyte cell-line upcyte. Several extracts used as dietary supplements increased activity in the DNI panel, most commonly through affecting AhR, PXR or Nrf2. Upon treatment of upcyte cells with these extracts, several clinically-relevant cytochrome P450s mRNA levels were modulated. Thus, this approach is a simple, mechanism-based way to examine the potential of botanical extracts and dietary supplements to cause drug-nutrient interactions.

2297 The Effect of Betulinic Acid on eNOS Expression via ERK5/HDAC5-Mediated KLF2 Pathway in Human Endothelial Cells

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Betulinic acid (BA) is a pentacyclic triterpene of the lupine-type that attenuates vascular diseases and atherosclerosis, but the mechanism by which it stimulates endothelial nitric oxide synthase (eNOS) is unclear. In previous studies, we demonstrated that beneficial cardiovascular effects such as increased NO production through enhancement of eNOS activity for this compound. Krüppel-like factors (KLFs) are a subclass of the zinc finger family of DNA-binding transcription factors, and are expressed in endothelial cells (ECs). Fluid shear stress induces KLF2 expression, which in turn regulates many flow-responsive genes, including endothelial eNOS. Furthermore, KLF2 and eNOS were found to regulate leukocyte adhesion to the endothelium by down-regulating expression of adhesion molecules that recruit leukocytes. Therefore, KLF2 and eNOS serve as genes that are important in the integration of multiple endothelial functions. The aim of this study is to investigate the molecular mechanisms of eNOS transcriptional activation by BA. In human EA.hy926 endothelial cells, BA increased the expression of eNOS and Krüppel-like Factor 2 (KLF2), which is a transcription factor for eNOS. In addition, BA induced both signaling pathways of CaMKIIα/AMPK/HDAC5 and ERK5/MEF2, which are involved in KLF2 expression and its transcriptional activity. Furthermore, treatment with a selective CaMKIIα inhibitor KN-62, a selective AMPK inhibitor Compound C and a selective inhibitor of ERK5 XMD8-92 suppressed BA-induced eNOS and KLF2 expression. Taken together, these results indicate that BA increase eNOS and KLF2 expression via the CaMKIIα/AMPK/HDAC5 and ERK5/MEF2 pathways. These findings provide further insight into the eNOS signaling pathways involved in the anti-atherosclerosis effects of BA.

2298 The Analgesic, Anti-Inflammatory, and Antipyretic Activities of the Bulb Extract of *Crinum jagus* (Amaryllidaceae)

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Pain, inflammation and sometimes fever are experienced in diverse pathologies and disease syndromes. Most of the medications used for treating these clinical entities including opioids, steroidal and non-steroidal anti-inflammatory agents do not only elicit adverse drug events due to toxicity but they are also expensive and not readily available especially in the rural settings of developing countries. Plants are known sources of bioactive compounds; there is indication to evaluate *Crinum jagus* bulb for highly acclaimed folkloric analgesic, anti-inflammatory and antipyretic effects. The extract was prepared by cold maceration in 80% methanol and concentrated in a vacuum rotary evaporator. Oral acute toxicity studies showed that methanol extract of *Crinum jagus* bulb (MECJB) was safe at 5000 mg/kg, hence did not cause mortality of experimental mice. The extract demonstrated a dose dependent inhibition of formalin-induced pain sensitivity in mice at 100, 200 and 400 mg/kg respectively. The lower test doses (100 and 200 mg/kg) of the extract exhibited a comparable anti-inflammatory effect to 50 mg/kg aspirin at 120 and 180 min in egg albumin-induced rat pedal edema. MECJB (100 mg/kg) exerted an almost equal potency with paracetamol (50 mg/kg) in suppressing yeast-induced hyperthermia. The extract reduced the mice rectal temperature by 79.5% relative to 83% with paracetamol. Two fractions (F_1 and F_2) with Rf values of 0.5 and 0.68 in hexane-ethyl acetate-chloroform (3:2:1) solvent system appeared to be the major analgesic components of the extract with ability to inhibit 90.2% and 80.9% acetic acid-induced pain sensitivity in mice respectively. Both fractions (F_1 and F_2) from the extract proved to have increased analgesic potency (85.8% pain inhibition) compared to piroxicam (50 mg/kg). *Crinum jagus* bulb could be a source for isolation of active compounds against pain, inflammation and fever.

2299 Effects of a 28-Day Oral Exposure to the Dietary Supplements Nattokinase and Lumbrokinase and of their Combined Exposure with Aspirin in Sprague Dawley Rats

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The dietary supplements nattokinase (produced by *Bacillus* subtilis var. *natto*) and lumbrokinase (powdered *Lumbricus rubellus* or *Eisenia fetida* earthworms) are promoted as beneficial for cardiovascular and circulation health. Both compounds have fibrinolytic activity and have been reported to degrade blood clots both *in vitro* and *in vivo*. We assessed whether an oral exposure to these compounds leads to an increased risk of bleeding, either when administered alone or in combination with aspirin. Adult male and female Sprague-Dawley rats were dosed orally for 28 consecutive days by gavage with the vehicle or 1,000 mg/kg body weight (bw)/day of nattokinase or lumbrokinase. Additional animals were exposed to these compounds in combination with aspirin (10 or 100 mg/kg bw/day). No treatment-related changes were observed in body weight, grip strength or motor coordination behavior assessments, bleeding time, or clinical pathology. As expected, both doses of aspirin inhibited the arachidonic acid-induced platelet aggregation. This effect was observed also when aspirin was co-administered with nattokinase or lumbrokinase, with the exception of the low aspirin plus lumbrokinase combination group, in which the platelet aggregation was similar to that in the vehicle group. Statistically significant effects were observed also in males treated with the high dose of aspirin for a few parameters measured by thromboelastography. The magnitude of these effects was more pronounced in the high aspirin plus nattokinase combination group; however, the main driver of the effect was aspirin. The only treatment-related histopathological lesions were an increase in the incidence of mucosal erosion and regenerative epithelial cell hyperplasia in the glandular stomach of the high aspirin groups, individually or in combination with lumbrokinase or nattokinase. The results suggest a lack of significant toxicity of nattokinase and lumbrokinase upon oral exposure. *This work was sponsored under an interagency agreement between the FDA/NCTR and the NIEHS/NTP (FDA IAG # 224-12-0003/NIEHS IAG # AES12013).*

2300 CBD Particle Size Dynamics: A Characterization of Cannabidiol E-liquids

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Despite the widespread use of electronic cigarettes and e-cigarette alternatives, knowledge of possible acute and long term health effects remain limited. This is particularly true in regards to a popular vape alternative, cannibidiol, or CBD. Several studies have characterized nicotine based e-liquids, yet to our knowledge no data have been published characterizing e-cigarette alternatives such as CBD. As e-liquids are transformed in the process of aerosolization, and subsequently inhalation, there is a need to characterize the size and composition of particles in these products. Particle size characterization can be a useful predictor to determine where CBD aerosols deposit and potentially react within the respiratory system. In this study, particle size characterizations were obtained and analyzed for 5 different aerosolized CBD e-liquids using a 7 stage In-Tox cascade impactor. CBD e-liquids were chosen based on ingredient list in order to give a good representation of products currently available on the market. Among products chosen were a high VG/PG blend, a pure CBD distillate, an MCT oil blend, a CBD and VG/PG blend, and a JUUL competitor blend. Products were aerosolized using a 3 port Electronic Cigarette Aerosolization Generator (ECAG). Our study found that particle size of aerosolized CBD vape liquids differed significantly from product to product. Of note, 60% of the mass for aerosolized liquid in the high VG/PG blend was over 2.85 µm, while pure CBD distillate showed over 90% of the mass to be less than 1.72 µm. CBD concentrations in the e-liquid and the airborne aerosols were also evaluated and differed by CBD product type. These results show that aerosol deposition may vary between products, and further analysis is needed to determine chemical compositions of unregulated CBD e-liquids. Thus, the observed product-dependent variability in exposure parameters must be taken into account in animal and *in vitro* assessments of the toxicity of CBD products.

2301 Virgin Coconut Oil Mitigates Oxidative Stress Induced by Sodium Fluoride *In Vitro* and *In Vivo*

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Sodium fluoride is a well-known environmental pollutant with pro-oxidant potential. Fluorosis due to fluoride intoxication through drinking water is a serious health concern globally. The fluoride-induced oxidative stress model in rodents has been widely used in pre-clinical experiments aimed at evaluating the antioxidant potential of natural products and pharmaceutical formulations both *in vitro* and *in vivo*. Virgin coconut oil (VCO) extracted from coconut kernel without subjecting to any mechanical means is an emerging functional food with reported pharmacological properties and health benefits. Herein VCO, its polyphenolic (VCO-PF) and VCO-PF minus Oil fractions (VCO-OF) were individually tested against the fluoride-exposed normal intestinal cells (IEC-6) as well as in mice to address their contribution in the documented antioxidant potential. Pre-treatment of VCO-PF (40 µg/mL) was found to be effective in bringing down the fluoride-induced cell death when compared to VCO (100 µg/mL) and VCO-OF fraction (60 µg/mL) as analyzed by the tetrazoliun (MTT) reduction assay. Further, sodium fluoride (3 mM) exposure increased the intracellular ROS in IEC-6 cells as measured by the uptake and intracellular hydrolysis of 2′,7′-dichlorodihydrofluorescin diacetate (H_2DCFDA) and subsequent oxidation of the product H_2DCF to DCF (collectively referred to as the DCF fluorescence method). VCO-PF significantly reduced the fluoride-induced ROS generation assessed based on the DCF fluorescence. VCO-PF, however, did not alter the ROS levels in normal cells, indicating its specific action towards the fluoride ions in the cellular milieu. In mice intoxicated with sodium fluoride in drinking water, oral administration of VCO (2 ml/Kg bw) as such reversed the drop in the hepatic catalase, glutathione-s-transferase, glutathione reductase and superoxide dismutase activities and maintained redox status to near normal levels. Concomitantly, hepatic lipid peroxidative changes as inferred by the thiobarburic acid-reactive substances were minimal in these animals. On the other hand, VCO-PF and VCO-OF fractions were found to be less effective in lowering the sodium fluoride induced increases in the hepatic oxidative stress markers. It is thus reasoned that oil components in VCO complement the natural antioxidant components for their better bioavailability.

2302 The Acute Toxicity of Water Hemlock (*Cicuta douglasii)* in a Goat Model

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Water hemlock (*Cicuta douglasii*) is one of the most toxic plants in North America. It has been reported to cause numerous poisonings in livestock species, as well as humans. Humans eat water hemlock because they mistake the tuber of water hemlock to be wild carrots or wild parsnips. Clinical signs in humans poisoned by water hemlock include nausea, sweating, salivation, vomiting, severe seizures and death. The toxins in water hemlock are C_{17} polyacetylenes, with cicutoxin being the most studied. The proposed mechanism of action of these toxins is due to their action as noncompetitive gamma aminobutyric acid ($GABA_A$) receptor antagonists in the central nervous system. Little information is known regarding the amount of tuber required for death to occur. Therefore, the objective of this study was to determine a lethal dose of water hemlock in a goat model. Tubers and above ground parts were dosed separately to goats via oral gavage of freeze-dried ground plant material. All goats dosed with tubers at 2.0, 0.5 and 0.25 g/kg developed severe seizures and were dead/euthanized by 30-120 min after dosing, with a clear dose-dependent response as to the severity and time of onset of the seizures. None of the goats dosed with tubers at 0.1 g/kg developed any clinical signs of poisoning. Similarly, none of the goats dosed with above ground parts of the plant at 2.0 g/kg showed any clinical signs. When goats were dosed with above ground parts at 10.0 g/kg, one goat developed seizures 2 hours post dosing and was euthanized. Two other goats showed minor signs of poisoning, while the fourth goat exhibited no adverse effects. The results from this study confirm anecdotal observations that the above ground parts of water hemlock are much less toxic than the tubers. The results from this study indicate that cicutoxin is the most toxic of the C_{17} polyactylenes, as the concentration of cicutoxin is much greater in the tubers compared to the above ground parts of the plant. These results also suggest that the NOEL for tubers in goats is 0.1 g/kg. This would correspond to 1-2 small fresh tubers.

2303 The Antimycobacterial, Cytotoxicity, and Selectivity Index of Some Menispermaceae Species

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Tuberculosis is a disease of worldwide occurrence that affects millions of people annually. One of the top 10 causes of death and the leading cause from a single infectious agent. Resistance is developing against current antituberculosis drugs, which also have toxicity concerns. Some plants from the Menispermaceae family have been used traditionally to treat tuberculosis and cough related symptoms. In this study, the antimycobacterial effect of *Cissampelos owariensis*, *Cissampelos mucronata* and *Tinospora fragosa* from the Menispermaceae family were investigated. Acetone, methanol:water (4:1), hot water and dichloromethane:methanol (1:1) extracts of *Cissampelos owariensis* whole plant, *Cissampelos mucronata* leaves, and stem, *Tinospora fragosa* aerial part were tested against non-pathogenic and pathogenic mycobacterial strains (*M. smegmatis*, *M. fortuitum*, *M. aurum,* and *M. bovis*) using a two-fold serial microdilution assay and thiazolyl blue tetrazolium bromide (MTT) as the growth indicator. Cytotoxicity of the active extracts was determined using a tetrazolium based colorimetric assay against Vero monkey kidney cells. The selectivity index of the active extracts was also evaluated. The acetone extracts were most active with minimum inhibitory concentration (MIC) values between 30 - 940 µg/ml. *Cissampelos mucronata* (leaves and stem) and *Cissampelos owariensis* were toxic to Vero cells with LC50 values of 20, 30, 40 µg/ml respectively while *Tinospora fragosa* was safer with LC50 of 3180 µg/ml. *Tinospora fragosa* also had the highest selectivity index of 39.75. Further studies are on-going on the isolation of the bioactive compounds of the extracts.

2304 Development of a Method to Survey Hemp-Derived Ingredients in Cosmetic Products

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As a result of the Agriculture Improvement Act of 2018 (the 2018 Farm Bill), which removed hemp from the Controlled Substances Act, the FDA observed an increase in the number of cosmetic products being marketed in the United States containing hemp-derived ingredients, including cannabidiol (CBD). In order to better understand the presence of these ingredients in cosmetic products, we initiated a project to develop an assay which would allow a survey of marketed products. We selected the products to be surveyed from a publicly available database (Mintel Global New Products Database or GNPD). Our search of the GNDP between 2017 to the present showed that, in addition to cosmetic products marketed in the United States, there appears to be an increase in cosmetic products claiming to contain hemp-derived ingredients internationally. The overall objective of this project was to develop a robust analytical method to assess the quality and quantity of hemp-derived ingredients used in marketed hemp-containing cosmetics. A total of 69 hemp and/or CBD containing cosmetic products in the GNPD were selected based on product labels stating what they contained. The presence of CBD, Δ9-tetrahydrocannabinol (Δ9-THC), and eight other marker cannabinoids found in *Cannabis sativa* were determined using a newly developed LC/MS method. Twenty-four out of 69 (34.8%) products were found to contain CBD. Of these, 19 products contained only CBD; whereas five also contained THC or THC plus other cannabinoids. All CBD positive products except for one had label information related to CBD, hemp extract, *Cannabis sativa* cell culture extract, phytocannabinoids, hemp or industrial hemp. Hemp seeds do not naturally contain significant amounts of THC or CBD. However, one product that claims to contain *Cannabis sativa* seed oil was also positive for CBD and THC. The remaining 45 (65.2%) cosmetic products did not contain CBD or any other cannabinoids evaluated in the assay. The products that tested negative for cannabinoids all claimed to contain *Cannabis sativa* seed oil (34/45) or hemp oil (9/45) in their list of ingredients.

2305 Creating a Literature Database for Cannabidiol (CBD): Systematic Evidence Mapping

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As part of the US FDA's evaluation of potential regulatory frameworks for cannabidiol (CBD) and other cannabis-derived products, the agency has consistently raised concerns about the limited scientific information on CBD and need for further study. To assist in identifying knowledge gaps and inform future decision making, systematic mapping of the safety-related literature for CBD was performed. The systematic evidence map approach uses a structured process to describe the state of knowledge for a topic and provides a comprehensive synthesis of available data that can be queried for specific information. The systematic map for CBD was prepared using evidence-based methods, following an *a priori* protocol and the PECO (population, exposure, comparator, outcome) approach to develop research objectives. A broad and transparent systematic literature search was conducted in PubMed and Embase. Titles and abstracts were assessed and categorized in DistillerSR, and evidence tables and other visualizations prepared. The literature search yielded a total of >3,100 unique returns; of these, >2,200 articles (most published since 2010) were deemed relevant and included in the systemic map. Studies evaluating the beneficial properties and mechanistic pathways of CBD accounted for ~40% and ~20%, respectively. Regarding safety, the frequency of endpoints of interest assessed was as follows: pharmacokinetics > drug interactions > developmental and reproductive toxicity (DART) = general toxicity > immunomuodulatory effects > mutagenicity/genotoxicity = hepatotoxicity. Additional categorical information was assessed, including test material, test system, route of exposure, and adverse/beneficial findings. For example, studies reporting DART outcomes were conducted primarily in laboratory animals; few were conducted *in vitro* or in humans. While many studies reported adverse reproductive effects associated with CBD, the lack of a published guideline compliant reproductive toxicity study was identified as a data gap. This assessment provides a reliable summary of the state of the evidence for evaluating health risks following exposure to CBD. The mapping results will help identify knowledge gaps and future research needs, as well as support future systematic reviews and other safety assessments of human exposure to CBD.

2306 Protective Effect of Extract of *Arrabidaea chica* Verlot (Bignoniaceae) on SH-SY5Y Cells Treated with Zearalenone Metabolites Mycotoxin

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Arrabidaea chica is a medicinal plant with numerous health benefits such as wound healing, immune system stimulation, and antioxidant properties, among others. Zearalenone metabolites (α-ZOL and β-ZOL) are mycotoxins with estrogenic capacity produced by some species of *Fusarium* that occur as contaminants, mainly in cereals. The aim of this study was to evaluate the protective effect of the ethanolic extract of *A. chica* leaves against the cytotoxicity of α-ZOL and β-ZOL in an undifferentiated neuroblastoma cell line, SH-SY5Y. Cells were cultured in 96-well plates for 24 h followed by 24 and 48 h treatment with the extract (4-1000 ppm). The cytotoxicity of β-ZOL and α-ZOL was measured in 9 concentrations in a range from 2 to 100 µM. The cytoprotective effect was evaluated using a mixture of concentrations below the IC50 of β-ZOL and α-ZOL, with concentrations of 16 and 32 ppm of the plant extract, respectively. Cell viability was evaluated by the MTT assay. Cells treated with the plant extract had viability greater than 50% at 63 ppm in the 24 and 48 h tests. Cells treated with α-ZOL remained viable during the first 24 h. However, at 48 h concentrations of 25, 50 and 100 µM of α-ZOL had viability below 50%. In addition, β-ZOL at 24 h showed cytotoxicity below 50% only at 100 µM while at 48 h viability decreased below 25% for 12.5, 25, 50 and 100 µM. A Mixture of *A. chica* extract at 32 ppm and α-ZOL showed a significant increase in cell viability at 25 and 50 µM ($p<0.05$). Mixtures of the plant extract and β-ZOL improve significantly cell viability at 48 h for 12.5, 25, 50 and 100 µM of β-ZOL ($p<0.001$). In short, extract from this plant isolated from the flora of the Colombian Caribbean is promising to counteract the toxic effects of mycotoxins that affect cereals. *Colciencias-UniCartagena (Grant FP 44842-212-2018, Scholarships 567-2012 and 727-2015), and Spanish Ministry of Education and Competitiveness (AGL2016-77610-R).*

2307 Evaluation of Levels of Select Toxic Metals in Commonly Used Herbal Medicines in Benin City, South-South Nigeria

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Herbal medicine has been gaining growing attention as medicinal plants are now the major remedy to diseases in some places, especially in Africa. Some herbal preparations are made without standard manufacturing and safety standards, a practice that makes the products susceptible to contaminants which may include toxic metals. Even at low concentrations or levels of exposure, toxic metals have also been reported to pose health risks to man. The study aimed to evaluate the levels of toxic metals in herbal medicines commonly prepared and used in Benin City, Edo state, Nigeria. Herbal medicines (n=8) were purchased from on-the-street vendors and evaluated for levels of five toxic metals (lead, nickel, mercury, cadmium and arsenic). Analysis of toxic metals was carried out in the analytical services laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan using Inductively Coupled Plasma Mass Spectrometry. The results obtained should that lead (mg/L) respectively detected in the eight sampled herbal preparations were 0.025, 0.022, 0.092, 0.088, 0.052, 0.074, 0.051 and 0.092; while nickel levels (mg/L) were 0.075, 0.066, 0.277, 0.265, 0.153, 0.158, 0.029, and 0.052; and levels of cadmium in mg/L detected were 0.014, 0.013, 0.052, 0.50, 0.029, 0.029, 0.153 and 0.277. In addition, Levels of mercury (mg/L) observed in the respective herbal samples were 0.035, 0.031, 0.129, 0.124, 0.071, 0.072, 0.071 and 0.129; and arsenic (mg/L) levels as observed were 0.002, 0.002, 0.007, 0.006, 0.004, 0.004, 0.004 and 0.007. Data obtained from this study indicate that the herbal preparations studied contain detectable amounts of toxic metals and by implication, the frequent and unorthodox use of these herbal preparations may increase the body burden of these metals which have been reported to be toxic at any level of exposure.

2309 Paraquat Inhalation, a Translational Route of Exposure, Results in Sex-Specific Alterations in Locomotor and Olfactory Behavior

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Many widely used pesticides, including paraquat (PQ), exhibit neurotoxic effects. Historically, oral, dermal or injection (s.c., i.p.) exposure to pesticides have been used to evaluate toxicity. However, increasing evidence suggests that pesticide inhalation - the realistic mode of human exposure - is an emerging air pollution concern. Epidemiological research indicates that agricultural environments are associated with Parkinson's disease (PD), a phenotypically heterogeneous disease that is hallmarked by bradykinesia as a result of loss of nigrostriatal dopaminergic neurons. Such motor symptoms are preceded by a myriad of prodromal symptoms including anxiety, loss of balance and hyposmia, which may serve as outcome measures for the early stages of PD. Previous research indicates that exposure to PQ via injection results in PQ accumulation in brain, dopaminergic cell loss, motor function alterations, and olfactory deficits in rodent models. Here, we utilized an environmentally-relevant whole-body inhalation paradigm to determine if similar PQ accumulation and neurobehavioral effects consistent with the trajectory of PD were observed. We exposed juvenile (PND40) male and female C57bl/6J mice to either filtered air or PQ aerosols generated using an ultrasonic nebulizer (200 µg/L for 4 hours per day) for 1 day only or for 7 days. An additional group of adult mice (PND120) was exposed to same concentration for 20 days. In all exposures, whole body inhalation resulted in PQ accumulation in olfactory bulb and cerebellum of exposed animals as determined by Q Exactive Plus mass spectrometry, but we did not observe any adverse pulmonary effects or weight loss in exposed mice. Females exposed to PQ for 7 days made significantly more errors in an odor discrimination test, which suggests that repeated exposure to inhaled PQ may adversely affect olfaction. Adult males exposed to PQ for 20 days exhibited significantly lower ambulatory episodes and stereotypic counts, suggesting prolonged PQ inhalation exposure results in locomotor deficits. These data indicate that low level whole body inhalation resulted in PQ accumulation in the brain and alterations to locomotor and olfactory behavior. These behavioral effects are sex-specific, and future research is needed to identify mechanisms of sex-differentiated toxicity. *Supported by ES001247 and ES025541.*

2310 An Inter-Species Comparison of Renal Transport of MCPA *In Vitro*

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4-Chloro-2-methylphenoxyacetic acid (MCPA) is a phenoxyacetic acid herbicide, a class of chemicals that shows species differences in toxicity, with dog being particularly sensitive compared to other species. Toxicokinetic measurements show prolonged systemic exposure in dog compared to other species. The widely accepted proposed mode of action is that dog has an intrinsically lower capacity to secrete some phenoxyacetic acids *via* the kidney, resulting in the observed higher and more prolonged systemic exposure. The aim of this study was to test this hypothesis using an established *in vitro* proximal tubule cell model, to compare the movement of [^{14}C]MCPA across kidney cell membranes in rat, dog and human. Primary dog, rat and human proximal tubule cells, cultured to form monolayers, were used to investigate the renal handling of MCPA in these species. The activity of the organic anion transporters OAT1 and OAT3 in the model was determined using the standard substrate, para-aminohippurate (PAH). Probenecid, a specific OAT1/3 inhibitor, was co-administered with MCPA or PAH to confirm the involvement of these transporter proteins. Secretory flux in all species was shown to be greater than absorptive flux, consistent with OAT1/3 transporters being a mechanism of excretion for MCPA. There were clear species differences in the renal transport of MCPA, with rat and human showing similar secretory flux, which was markedly lower in dog. Data from this assay therefore confirm that the secretory flux of MCPA is mediated by an organic anion transporter, with OAT1 and/or OAT3 identified as the predominant transporters, implying that MCPA has lower affinity for dog isoforms, than for rat and human. The data provides mechanistic evidence for the observed differences in renal handling of MCPA, demonstrating similarity between rats and humans, whilst highlighting the poorer excretory efficiency in dog. These findings explain the significantly slower clearance of MCPA measured in dog compared to rats and humans, and provide a mechanistic explanation for the greater sensitivity of dogs to MCPA toxicity, supporting the position that dog is not an appropriate species to be used for human risk assessment.

2311 High-Throughput Screening Data for the Conazoles Do Not Support a Human-Relevant Common Mechanism Group for Cumulative Risk Assessment

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Cumulative risk assessments (CRAs) require the establishment of a common mechanism group (CMG), which consist of a group of pesticides that share a common mechanism of toxicity. To this end, adverse outcome pathways (AOPs) can be used to examine whether a CMG exists in humans. The conazole fungicides have recently been considered for a CRA based on liver effects, but multiple lines of evidence indicate the known liver AOP for the conazoles is only operant in mice and rats, and not in humans. However, high-throughput screening data offer additional mechanistic information, and it is important to consider this information in the overall weight of evidence analysis. Thus, the primary objective of this work was to determine whether high-throughput screening data in ToxCast/Tox21 support the current AOP for the conazoles. ToxCast/Tox21 data for the conazoles were analyzed following published methods to: (1) Examine potential constitutive androstane receptor (CAR) bioactivity (i.e., molecular initiating event in AOP), (2) Explore other potential bioactivities, (3) Determine whether quantitative structure-activity relationships exist between specific conazole ToxPrint chemotypes and assay bioactivity. It was found that the conazoles were not active in ToxCast/Tox21 CAR assays (i.e., ATG_CAR_TRANS_up, TOX21_CAR_Agonist) after controlling for cytotoxicity and quality control flags. When additional ToxCast/Tox21 assays were considered, the conazoles had distinct bioactivity profiles, and there was more bioactivity in assays with rat targets than those with human targets. Finally, binary correlations (Phi coefficients) were calculated between conazole ToxPrints (i.e., ring:hetero_[5]_N_triazole_(1_2_4-), ring:hetero_[5]_N_triazole_(1_3_4-), ring:hetero_[5]_Z_1_2_4_1_3_4-Z) and ToxCast/Tox21 assays, and it was found that these ToxPrints were strongly associated with rat CYP inhibition, but not with human CYP inhibition. Thus, high-throughput screening data did not identify a human-relevant CMG for the conazoles, and therefore a CRA is not appropriate.

2312 Localization of Accumulation of Glyphosate Herbicide within the Body of *Drosophila melanogaster*, a Nontarget Animal

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The widespread use of glyphosate-containing herbicides like Roundup in food and crop production has raised questions about whether their presence in foods and the environment can negatively impact public health. Glyphosate-containing herbicides, designed to destroy agricultural weeds, are extremely toxic to living organisms at high concentrations. Although Roundup manufacturers claim the herbicide formulations are non-toxic, growing evidence indicates that animals and humans exposed to high concentrations suffer a variety of health consequences, including development issues, reduced fertility, and disease. Our preliminary data suggest that Roundup increases mortality, affects ovary size, and can accumulate to high concentrations in *Drosophila melanogaster.* This study aims to determine where glyphosate is accumulated within *Drosophila* to begin to explore its mechanism of action in the physiological systems of non-target organisms. In this study, we exposed *Drosophila melanogaster* to medium containing Roundup Super Concentrate with a glyphosate concentration of 10g/L to localize glyphosate accumulation within the organism. A total of 20 flies chronically exposed to the treatment for 7 days as adults were dissected to isolate their heads, thoraxes and abdomens. Separately, both ovaries or both testes were removed from 10 females and 10 males and tested for glyphosate accumulation. The samples were analyzed by HRI Labs by liquid chromatography-mass spectrometry to determine their glyphosate concentration. The ovary and testes samples contained the least amount of glyphosate, displaying concentrations of 614.2 µg/g and 211.6 µg/g. The highest amount of glyphosate was accumulated within the abdomen with a concentration of 4572.1 µg/g, followed by the thorax at 1577.5 µg/g and the head displayed a final glyphosate concentration of 1100.8 µg/g. That lower levels of glyphosate were accumulated in gonad samples suggests that the reproductive effects of glyphosate exposure are not a result of glyphosate affecting the reproductive organs, but rather an indirect mechanism. An overall high concentration of glyphosate within the abdomen may indicate undigested food remained within the digestive tract of the flies. Accumulation of glyphosate within the head may be linked to growth, developmental and reproductive effects as the neuroendocrine system in the brain regulates the production of hormones and other biological processes. Along with ongoing experiments, this sheds light on how glyphosate-containing herbicides affect the health of those exposed to these herbicides throughout their lifetime.

2313 Safety Assessment Case Study of an Herbicide Groundwater Metabolite Using Realistic Exposure Route and Dose Setting Approach

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Carfentrazone-ethyl is a protoporphyrinogen oxidase (PPO) inhibitor herbicide undergoing EU renewal. Carfentrazone-ethyl is not classified for acute, genotoxicity, reproductive/developmental toxicity or carcinogenicity. M9 is a soil metabolite which was not identified in the rat metabolism studies. The predicted potential exposure concentration in groundwater (PECgw) of M9 exceeded 0.75 µg/L in a couple EU scenarios with the highest PECgw of 0.885 µg/L. According to the Sanco/221/2000 groundwater metabolite guidance document (rev. 10, 2003), a non-relevance assessment is triggered including herbicidal activity, acute oral, genotoxicity, repeated dose toxicity studies and consumer risk assessment. The studies demonstrated that M9 is less than 50% herbicidally active compared with the parent; not acutely toxic (oral LD_{50}>5000 mg/kg); not genotoxic in a battery of *in vitro* and *in vivo* genotoxicity studies. In the 28-day oral toxicity study in rats, M9 was administered *ad libitum* via drinking water (a more realistic method of exposure for consumers) at concentrations of 1, 10, and 100 ppm (near the M9 solubility limit). The NOAEL was determined to be 100 ppm, equivalent to 10 and 13 mg/kg/day for males and females, respectively. An acceptable daily intake (ADI) of 0.01 mg/kg/day was derived using this NOAEL of 10 mg/kg/day and a safety factor of 1000x. A consumer risk assessment showed that maximum theoretical consumption of M9 via drinking water contributed 0.30%, 0.89%, and 1.32% of the ADI for adults, toddlers and bottle-fed infants, respectively. Based on a conservative 10% allocation of the total daily intake to drinking water, consumption of M9 via drinking water will not represent an unacceptable risk to consumers. This case study exemplifies a suitable safety assessment approach for ground water metabolites based on toxicology studies that use a realistic human exposure approach (i.e., drinking water tested up to the limit of solubility in contrast to oral feeding of solid test materials at extremely high dose levels).

2314 Monitoring of Agricultural Workers Exposed to Cholinesterase-Inhibiting Pesticides

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The California Medical Supervision program is intended to protect workers who regularly mix, load, or apply Category I and II organophosphate (OP) and N-methyl carbamate (CB) pesticides. Exposure to OPs or CBs lower the level of cholinesterase (ChE), a critical enzyme that regulates neurotransmitter signaling. The Program uses ChE levels in blood as a biomarker to detect excessive exposure to these pesticides prior to the onset of illness. Since 2011, the six clinical laboratories performing ChE analysis under this Program have been required to report test results electronically to the California Department of Pesticide Regulation and the California Office of Environmental Health Hazard Assessment (OEHHA), allowing for statewide evaluation of the Program. Analysis on data collected from 2011 through 2013 revealed that poor data quality and missing information significantly hampered data analysis. This led to regulatory changes, effective January 2017, requiring employers of workers subject to the Program's requirements to exclusively contract with medical supervisors (physicians) who are registered with OEHHA. In the last three years, 120 physicians have registered with OEHHA. Preliminary analysis of data from 2017 - 2018 found a total of 16,719 unique records, of which 12,739 (76.19 %) were reported by registered physicians. Overall, registered physicians were more likely to order 14-day baseline tests (44.64% vs. 12.00%) and follow-up with their patients over time (21.81% vs. 3.51%). All test results of significant ChE depression (<80% baseline) were from individuals followed by registered physicians (19 of 327 individuals with identifiable baseline estimates had at least one reported event of significant ChE depression), which may have been due to the higher frequency of follow-up tests conducted by registered physicians. In conclusion, we observed an improvement in data quality with the registration of physicians, which enhanced data analysis, helped to increase the accuracy of our Program evaluation, and most likely improved the effectiveness of the Program to protect pesticide handlers from over-exposure to OPs and CBs.

2315 Occupational Organophosphate Pesticides Exposure and Reproductive Effects in Women Farmers

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Since chayote sowing is perennial, in Veracruz (as in the whole country), many people are chronically exposed to pesticides. Most pesticides act as endocrine disruptors, thus compromising reproductive function in humans. Luteinizing hormone (LH) is a gonadotropic hormone, responsible for regulating reproductive function in mammals, as well as exerting primary actions on gonads and its blood concentration is considered an infertility marker. The aim of the study was to determine the relationship between serum luteinizing hormone and occupational exposure to organophosphate pesticides in Veracruz farmers. This case-control study was conducted on farmers and residents of Cuautlapan, Veracruz, Mexico. Information related to pesticides exposure and clinical symptoms were collected from a questionnaire. Serum LH levels were determined by the ELISA method. All analyses were conducted in the statistical package STATA v.15.0., using a significance of $p<0.05$ value. In total of 64 subjects were included in the study, with an average age of 34 years. 51.5% of the population was exposed to pesticides (14 women and 19 men), mainly to chlorpyrifos (25.6%) and methamidophos (16.3%), of which 33% do not use protective equipment. Headaches, blurred vision, nausea, insomnia and pruritus were the most prevalent symptoms of intoxication. In woman LH levels were found outside the reference values according ovulation phase and menopause ($p<0.05$) in exposed group. Occupationally exposed men had higher levels of LH compared to those not exposed ($p<0.05$). It is concluded that chronic exposure to organophosphorus pesticides affects the fertility of men and women. However, women have more health problems due because luteinizing hormone modulates their reproductive phase and menopause.

2316 Residential Proximity to Agricultural Crops and Acetylcholinesterase Activity, a Pesticide Exposure Biomarker, Assessed from Childhood through Adolescence

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Off-target drift of pesticides from farms increases the risk of pesticide exposure of people living nearby. Commonly used insecticides in agriculture are cholinesterase inhibitors (i.e. organophosphates and carbamates), which inhibit the enzymatic activity of acetylcholinesterase (AChE). AChE is a known regulator of the neurotransmitter acetylcholine. In this study we characterized the associations between residential proximity to greenhouse agriculture where pesticides are used and AChE activity in children (lower values indicate greater cholinesterase inhibitor exposures). This study included 1145 observations from 3 exams (2008, Apr 2016 and Jul-Oct 2016) of 622 children aged 4-to-17 years living near agriculture in Ecuador (ESPINA study). AChE and hemoglobin were measured from fingerstick blood samples. Geographic positioning of greenhouses (primarily floricultural) and homes were obtained using GPS receivers and satellite imagery. We calculated the distances between homes and the nearest greenhouse edge and areas of greenhouse crops within various buffer zones around homes ranging from 150m to 500m. Repeated-measures regression models adjusted for hemoglobin and demographic, socio-economic and anthropometric variables. The pooled mean (SD) of AChE activity was 3.58 U/mL (0.60). The median (25^{th}-75^{th} %tile) of residential distance to homes was 334 m (123, 648) and of crop areas within 500 m of homes was 18,482 m^2 (7115, 61841, among those with non-zero values). Areas of crops near homes was negatively related to AChE activity. This association was strongest among children living within 150m of crops (AChE difference per 10,000 m^2 [95% CI] = -0.07 U/mL [-012, -0.01]), and weaker, but also significant, associations were observed among those living within 151-500m of crops. Overall, residential proximity to the nearest crop was not associated with AChE activity (AChE difference per 100m of *proximity*= 0.000 U/mL[-0.003, 0.005]); however, this association was significant among children who had at least 811m^2 (5^{th} percentile, N_{obs}=522) of crops within 300m of their homes (-0.051 U/mL [-0.098, -0.004]). Our findings suggest that residential proximity to greenhouse crops where pesticides are used, and greater areas of such crops near homes result in sufficient pesticide exposures to decrease AChE activity of children living within 500m (and especially 150m), despite the relative confinement of greenhouse agriculture. Added precautions to reduce the off-target drift of pesticides from crops, or more stringent requirements separating residential from agricultural zoning are recommended.

2317 Immune Effects of Carboxylesterase Inactivation in the Neonatal Murine Lung

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Carboxylesterases (Ces) are enzymes that metabolize xenobiotics and anti-inflammatory lipid mediators. Chlorpyrifos (CPF) is an organophosphate pesticide (OP) that inhibits Ces activity at doses that do not inhibit acetylcholinesterase, the canonical toxic mechanism of action. Our lab's recent data showed that chronic low-dose CPF inhibited lung Ces1c and Ces1d in neonatal mice. We hypothesized that pretreatment of neonatal mice with CPF would attenuate LPS-induced lung inflammation because of the diminished catabolism of anti-inflammatory lipids. Neonatal mice were treated with CPF (2.5 mg/kg, p.o.) or corn oil for 7 days from post-natal days 10 to 16, followed by LPS (1.25 mg/kg, i.p.) or saline. Tissues were harvested 6 hours after the LPS treatment. Lung *IL-6*, *TNF-α*, and *IL-1β* mRNA levels in response to LPS were unaltered by CPF exposure. CPF also did not alter the innate and adaptive immunophenotype of lung tissue in response to LPS. On the other hand, CPF decreased lung 2-arachidonoylglycerol (2-AG) levels independent of LPS treatment, but it did not alter the levels of other lipid mediators including prostaglandins. Contrary to our hypothesis, Ces inhibition by CPF did not modulate LPS-induced lung inflammation, and 2-AG levels were decreased rather than increased. These results suggest that Ces1c and 1d are bio-scavenging enzymes that have a protective role against CPF exposure in neonatal lung. *Supported by NIH R15 GM128206.*

2318 Effect of Calcium and Pyrethroids on Adipogenesis and Lipid Accumulation in Murine Adipocytes

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Pyrethroids are a chemical class of insecticides that are used by pest management professionals. They are structurally derived (manmade) from naturally occurring insecticides called Pyrethrins. Pyrethrins are a mix of six chemicals used to control mosquitoes, fleas, ants and, other pests. Two pyrethroids were indicated as possible obesogens in a blinded study of 70 chemicals in mouse 3T3-L1 cells, therefore an investigation of the several pyrethroids on the market and their two common metabolites was initiated. This study was developed to determine the mode of action(s) for pyrethroids in 3T3-L1 cells across a dose range and in two different calcium concentrations. In 96 well plates, 3T3-L1 cells were treated with two different concentrations (1.8mM and 10mM) of Calcium, three different concentrations (1μM, 5μM, and 10μM) of 10 separate pyrethroids, vehicle (0.05% DMSO) and rosiglitazone (1μM) as a positive control, in differentiation media, for 6 days in 96 well plates. On Day 6, the cells were fixed with 4% Paraformaldehyde and stained no more than 24 hours prior to ImageXpress® Micro (IXM) analysis with Nile Red and Hoechst 33342. The experiments were repeated in three biological replicates. Data were analyzed using linear mixed-effects models to account for multiple images obtained per well, and technical replicate wells within a plate. Biological replicates were defined as the same experimental conditions tested on a different multi-well plate on separate days. For each pyrethroid, cell count (to indicate cytotoxicity), number of adipocytes, and the mean lipid droplet area (MLDA) (μm^2) per adipocyte were evaluated using this mixed effect model approach. There was no significant decrease in cell count for Cypermethrin, Bifenthrin, Allethrin, or Tetramethrin, and no interaction with calcium for cell count. There was no change in number of adipocytes or MDLA per adipocyte for any concentration of Bifenthrin, Allethrin, or Tetramethrin, and no interaction by calcium concentration. However, there was a significant increase in number of adipocytes and MDLA per adipocyte after exposure to 10μM Cypermethrin. 10μM Cypermethrin increased the number of adipocytes by 9% and increased the MLDA per adipocyte by 17.5%. These data suggest that Cypermethrin had an effect on adipogenesis and lipid accumulation, regardless of calcium concentration.

2319 Assessment of Human Health Risks from Potential Exposure to the Pesticide Chlorthal-Dimethyl and Its Degradates in California Groundwater

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The Office of Environmental Health Hazard Assessment (OEHHA) conducted a risk assessment for the pesticide active ingredient chlorthal-dimethyl (DCPA; dimethyl tetrachloroterephthalate; dacthal) and its environmental degradates, monomethyl tetrachloro-terephthalic acid (MTP) and tetrachloroterephthalic acid (TPA). DCPA is a pre-emergent herbicide used for the control of annual grasses and certain broadleaved weeds. TPA and MTP were detected in multiple water wells in three California counties by the Department of Pesticide Regulation's (DPR) groundwater monitoring program. The highest detections were 159 ppb for TPA and 0.13 ppb for MTP. OEHHA reviewed the available toxicity database to (1) evaluate DCPA, MTP, and TPA for toxicity endpoints related to carcinogenicity, mutagenicity, teratogenicity, and neurotoxicity, (2) develop public health concentrations (PHCs) in the drinking water for DCPA and TPA, and (3) use the PHCs to determine the human health risks from potential exposures in the drinking water. PHC is the concentration of a chemical in drinking water that is not expected to pose a significant risk to human health when consumed over a lifetime. The toxicological database was complete for DCPA, limited for TPA, and insufficient for MTP. OEHHA determined that the two degradates are more water soluble with much lower toxicities than their parent compound. Thus, separate PHCs for DCPA and for TPA were derived. A PHC could not be calculated for MTP due to lack of data. The lowest PHC was 2 ppb for DCPA, based on hepatocellular adenomas and carcinomas in mice following chronic dietary exposure. The PHC for TPA was 2500 ppb, based on no observable adverse effects in a subchronic rat dietary study, soft stools and hematologic effects in a rat subchronic gavage study, and applicable uncertainty factors. Because MTP is an intermediate degradate of DCPA, the PHC for TPA was applied to the sum of the highest detected levels of TPA and MTP for the assessment. OEHHA determined that neither degradate is likely to cause adverse health effects if found in the groundwater at the levels detected, and consumed in drinking water over a lifetime. The highest detected levels of MTP and TPA were more than 15-fold lower than the calculated PHC, indicating an adequate margin of safety.

2320 Oral Exposure to Mancozeb Alters Serum and Feces Essential Metal Levels and Glutathione in Sprague Dawley Liver and Kidney

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Mancozeb (MZ), a member of the ethylene bisdithiocarbamate (EBDC) class of fungicides, is widely used commercially. MZ is complexed to Mn and Zn. The presence of metals in MZ, as well as the transchelation ability of EBDCs, raises the concern that MZ exposure produces metal overload-induced oxidative stress. Nabam (NB; sodium salt of EBDC) or a mixture of $MnCl_2$ and $ZnCl_2$ (MnZn) treatments distinguish MZ effects mediated by the EBDC backbone or metals. Our previous work found that MZ and NB caused significant accumulation of Cu in kidney of treated rats, but MZ caused a significant decrease in Cu in the rat liver. In both liver and kidney, Mn levels were increased in MnZn and MZ treated rats, but NB treated rats had decreased Mn levels. The aim of the present study is to use inductively coupled plasma-optical emission spectroscopy (ICP-OES) to measure Mn, Zn, Cu, and Fe levels in serum, blood clot, urine, and fecal samples from MZ , NB, and MnZn treated rats, as well as assess oxidative stress parameters (TBARS and total glutathione) in rat liver and kidney due to altered metal levels. Sprague-Dawley rats were treated orally with MZ, NB, MnZn, or vehicle control (25% (v/v) PEG400 : H_2O) every day for 28 days. After the last dose, rats were individually housed and fasted overnight with water *ad libitum* in metabolism cages. Urine and feces were collected and stored until further use. Rats were anesthetized and blood was collected via cardiac puncture. Coagulated blood was centrifuged to separate serum from the blood clot, and each were stored until analysis. Organs were harvested and stored in liquid N_2 until biochemical analyses. In urine and blood clot samples, there were no significant differences in the levels of metals tested in the treatment groups. Serum iron levels were significantly higher in NB (22.20 ppm/g) treated rats than in PEG (18.12 ppm/g) or MnZn (17.69 ppm/g) groups. MnZn and MZ treated rats had higher fecal Mn (140.4 and 134.7 ppm/g) and Zn (13.74 and 15.26 ppm/g) levels compared to PEG (Mn: 51.04, Zn: 10.75 ppm/g), but only MZ treated rats had decreased fecal Fe levels (78.64 ppm/g) compared to PEG (133.4 ppm/g). For all treatments, liver and kidney indicated no significant differences in TBARS levels, but total glutathione levels for NB and MZ treated rats were increased compared to PEG rat liver and kidney. Results demonstrate metal alterations in all treatment groups and shift in antioxidant balance in liver and kidney of NB and MZ treated rats.

2321 Mancozeb Exposure Results in Apoptosis and Disruption of Mitochondrial Complex IV and V Activity in Transformed Human Colon Cells

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Ethylene bisdithiocarbamate pesticides (EBDC), such as Mancozeb (MZ), are mainly used in agriculture as fungicides on a variety of crops. Structurally, this class of pesticide has an ethylene bisdithiocarbamate organic backbone complexed to metal moieties. Mancozeb is complexed to the metals manganese and zinc. Common routes of Mancozeb exposure are ingestion of food containing pesticide residue and occupational exposure. Previous work in our laboratory has demonstrated that Mancozeb exposure to both transformed human colon cancer cells and non-transformed human colon cells causes increased lipid peroxidation, decreased ratio of reduced glutathione to glutathione disulfide, and increased caspase 3/7, 8, and 9 activities. It was determined that both the ethylene bisdithiocarbamate backbone and metal moieties contribute to the toxicity of Mancozeb (Hoffman and Hardej, 2012; Hoffman et al., 2016). This study investigated the effect of Mancozeb on the induction of apoptosis and mitochondrial activity of HT-29 colon cancer cells. Previous experiments have shown cytotoxicity, induction of both apoptosis and oncosis, and decreases in both Complex I and Complex II enzyme activities. Current experiments utilized kinetic assays to evaluate and compare the effect of Mancozeb on the activity of mitochondrial enzymes, Complex IV and Complex V. Cells were dosed for 24 hours with MZ concentrations of 60 µM, 100 µM, and 140 µM. Mitochondria were isolated using a commercially available kit. Complex IV enzyme showed a significant decrease in activity at 60 µM, 100 µM, and 140 µM Mancozeb. Activity of Complex V enzyme showed a significant decrease at 60 µM Mancozeb. Scanning electron micrographs of cells exposed to Mancozeb (60-160 µM) for 24 hours showed a disruption of the plasma membrane, particularly blebbing. The current data indicates that HT-29 colon cancer cells exposed to Mancozeb results in decreased activity of mitochondrial Complex IV and Complex V.

2322 Epigenetic Modification by Mancozeb in Astrocytes and PC12 Cells

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Chronic exposure to manganese is associated with various neurological disorders such as Parkinson's disease. Mancozeb which contains manganese can potentiate parkinsonian toxicant MPP^+ toxicity and also activate NFκB in PC12 cells. Studies show that epigenetic related proteins JMJD3 and HDAC1 can regulate NFκB signaling pathway. Epigenetic modification in manganese neurotoxicity is not fully unveiled. This study was to elucidate the effects of mancozeb (MZ) and manganese chloride ($MnCl_2$) on epigenetic modification in astrocytes and PC12 cells. Epigenetic related protein DNMT1, JMJD3 and HDAC1 were the main focuses of this study. Cells were treated with MZ or $MnCl_2$ at 5 µM, 25 µM, and 50 µM for 24 hours. Nuclear proteins were extracted after chemical treatments. Protein concentrations were determined by Bio-Rad DC assay. Epigenetic related proteins (DNMT1, JMJD3 and HDAC1) and NFκB proteins were detected by using immunoassay kits from EpiGentek and Abcam, respectively. The results showed the levels of DNMT1 and HDAC1 were increased after MZ treatments in a dose-dependent manner in PC12 cells, but no change in the expression of JMJD3. In PC12 cells treated with $MnCl_2$, the levels of DNMT1, HDAC1 and JMJD3 were not significantly altered as compared with control. In astrocytes, only the levels of HDAC1 were increased in response to MZ and $MnCl_2$ in a dose-dependent manner. There were no changes in the levels of DNMT1 and JMJD3 of astrocytes after chemical treatments. Interestingly, MZ at 5 µM enhanced the expression of NFκB in PC12 cells, but inhibited the expression of NFκB at higher MZ doses. $MnCl_2$ has no effect on the NFκB expression in PC12 cells. For the expression of NFκB in astrocytes, MZ and $MnCl_2$ both decreased the expression of NFκB in a dose-dependent manner. And MZ has stronger effect on the expression of NFκB in astrocytes as compared with astrocytes treated with $MnCl_2$. Further studies are needed to uncover the relationship between the expression of epigenetic related proteins and NFκB in both cell lines in response to MZ and $MnCl_2$.

2323 Methylation of the Dithiocarbamate Fungicide Maneb Reveals Potential Toxic Mechanisms in Neuroblastoma

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The dithiocarbamate fungicide maneb (MB) has received research interest due to the increasing concern of the negative health effects of pesticides, as well as its association with Parkinson's disease (PD). However, few studies focus on the molecular mechanisms of MB in human cells, with identified toxicities including mitochondrial dysfunction, proteostasis disruption, and apoptosis of dopaminergic neurons. Our laboratory has previously reported distinct phenotypic changes of neuroblastoma cells exposed to acute, subtoxic levels of MB including decreased mitochondrial respiration, altered lactate dynamics, and metabolic stress. In this study, we aimed to define specific molecular mechanisms of MB through the comparison of several thiol-containing compounds and their effects on energy metabolism and antioxidant defense. The Seahorse XFe96 extracellular flux analyzer was employed to evaluate alterations in energy metabolism of SK-N-AS human neuroblastoma cells after acute exposure of an array of chemicals including dithiocarbamates (maneb, nabam, zineb) and other thiol containing small molecules (glutathione, N-acetylcysteine). These studies revealed MB and its methylated form (MeDTC) as unique toxicants, with significant alterations to mitochondrial respiration, proliferation, and glycolysis. Next, we investigated glutathione and thioredoxin/peroxiredoxin redox homeostasis, finding decreased glutathione and altered thiol oxidation status of peroxiredoxin 3 (Prx3, mitochondrial) after acute MB exposure. We then used redox Western blotting to pinpoint a potential MB-specific modification of cellular and recombinant Prx3, which we subsequently confirmed by Mass Spectrometry (MS) techniques. Finally, an ELISA for S-adenosylmethionine (SAM) revealed a MB-mediated decrease cellular SAM after a 2 hour exposure, confirmed by MS. The data presented strengthens the argument that MB can preferentially target mitochondrial enzymes containing thiol or iron-sulfur moieties, giving further credence to the MB model of PD in which many disease associated proteins contain these functional groups (Ubiquinol-cytochrome c reductase, aconitase, ALDH2, etc.). Additionally, our data supports a novel cellular "detoxification" mechanism via enzymatic methylation, potentially contributing to the complex toxicity profile of MB.

2324 Reduction of Pesticide Bioavailability with Charcoal and Clay-Based Sorbents

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Widespread use of pesticides has resulted in the accumulation of pesticide residues in the environment due to their persistence and stability. To reduce potential exposures, we have developed broad-acting clay-based materials that can be used to reduce the bioavailability and toxicity of diverse chemicals. In this study, activated charcoal (AC), calcium montmorillonite clay (CM) and acid-processed calcium montmorillonite clay (APM) were used to determine their potential as sorbents of dieldrin, an organochlorine insecticide, and glyphosate, an organophosphorous herbicide. We used adsorption isotherms, thermodynamics, and dosimetry studies to determine the capacities and affinities of the clays, enthalpies of the binding reactions, and potential doses of sorbent that could protect against high exposures. Cultures of *Hydra vulgaris* were used to determine the ability of sorbents to protect a living organism from pesticide toxicity. Additionally, soil and plant models were used to determine sorbent ability to reduce pesticide bioavailability in soil and to reduce plant uptake of pesticides from soil. Adsorption isotherms for all sorbents of dieldrin fit a Langmuir model. Isotherm results for APM showed high enthalpy (suggesting chemisorption) and high capacity (Q_{max} = 0.45 mol kg^{-1}), indicating tight binding of dieldrin. Inclusion of CM and APM resulted in the highest reduction of dieldrin toxicity (60 and 70%, respectively) in the hydra. Further work indicated that AC, CM and APM can significantly reduce the bioavailability of dieldrin from soil ($p \leq 0.01$). Inclusion of 1% AC and CM significantly reduced residues of a toxic glyphosate metabolite (AMPA) in corn sprouts (60%), confirming that these sorbents can reduce uptake of glyphosate from soil and water. These results suggest that the tested charcoal and clays can be effective sorbents of dieldrin and glyphosate and may be included in the diet and/or garden soil to protect against environmental exposures. These sorbents and combinations of sorbents can be further developed to bind other environmentally relevant chemicals and chemical mixtures. *Supported by NIEHS SRP P42 ES0277704.*

2325 A Tiered Approach to Prioritizing Registered Pesticides for Potential Cancer Hazard Evaluations: Implications for Decision Making

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The US has >800 pesticides (including insecticides, rodenticides, and herbicides) registered for use. Some historical pesticides (e.g., lindane, DDT) have been identified as human carcinogens and subsequently severely restricted or banned. A growing body of animal and human studies raise concern that some replacements may also be carcinogenic, including some high-volume pesticides with a potential for ubiquitous US exposure. The US Environmental Protection Agency (EPA) conducts cancer hazard evaluations of these chemicals, mostly during the pesticide registration process. These evaluations often rely on proprietary studies and can sometimes be outdated. We aim to examine the scope of registered pesticides in the US to identify gaps and to determine which non-banned pesticides would be potential candidates for a cancer hazard evaluation. A systematic, tiered review approach was used to prioritize pesticides currently listed in EPA's public database. Initial criteria for consideration include: (a) EPA's human carcinogenicity classifications; (b) high volume use in US agricultural, commercial, or residential sectors; (c) no recent carcinogenicity evaluation; and (d) pesticides not listed by the International Agency for Research on Cancer (IARC) or the US Report on Carcinogens (RoC). Subsequently, evidence maps for each pesticide were created from searchable libraries of peer-reviewed literature compiled since the last EPA carcinogenicity evaluation. Database searches focused on identifying epidemiological studies that investigated the relationship between human exposure to pesticides and cancer. Preliminary results identified 16 pesticides classified as possible or probable carcinogens by EPA, used in high volumes, not listed by IARC or RoC, and having no recent carcinogenicity evaluation by EPA, some for as many as 35 years. For six pesticides, including mancozeb, carbaryl, and metolachlor, multiple population-based cancer epidemiology studies with reports on at least three cancer-specific sites have been published since EPA's last review. These results warrant further scoping to determine if a re-evaluation is needed. We recommend prioritizing and updating hazard evaluations for these pesticides to ultimately inform regulatory and public health decision-making.

2326 Imidacloprid Containing Mesoporous ZnO/SiO_2 Nanopesticide as an Environmentally Friendly Controlled Release Formulation: An Approach Toward Sustainable Agriculture

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Poor utilization efficiency of pesticide formulations is one of the significant problems conjoined with conventional agronomic practices which have resulted in overuse of pesticides, leading to higher costs and ecological hazard not only to non-targeted organisms but also to humans. Pesticides related risks have also impacted adversely to flora and fauna as well as our aquatic systems. Recent innovations in nanotechnologies and consequently, its applications in agriculture towards nanopesticide formulations hold promise to address this challenge by improving the utilization efficiency of pesticides with a much lesser pesticide load. In the present study, a slew of unique nanopesticide controlled release systems (CRS) were designed by entrapping Imidacloprid, a neonicotinoid systemic insecticide, in mesoporous ZnO/SiO_2core-shell nanoparticles. These systems were characterized using microscopy, spectroscopy, and thermal techniques. Additionally, to further minimize the pesticide burden, a biopesticide tomatine was loaded in the CRS eliciting a synergistic effect in terms of insecticidal efficiency towards *Aphis craccivora*, a model target pest. Preliminary data purport photostability and controlled release behavior. Risk exposure study using human dermal fibroblast (HDF) cell line and *Vigna radiata* as a model plant delineated its safer toxicity profile when compared to the bulk pesticide. Future work will encompass ecotoxicology study evaluated as per OECD guidelines, on zebrafish, one of the non-target organisms, to assess any risk involved. The present work embodies the concept of better pest control and crop management using sustainable eco-farming approach.

2327 Chlorpyrifos-Induced Cell Proliferation in Human Breast Cancer Cell Lines Differentially Mediated by Estrogen and Aryl Hydrocarbon Receptors and KIAA1363 Enzyme After 24-Hour and 14-Days Exposure

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Organophosphate biocide chlorpyrifos (CPF) is involved with breast cancer. However, the mechanisms remain unknown. CPF increases cell division in MCF-7 cells, by estrogen receptor alpha (ERα) activation, although it is a weak ERα agonist, suggesting other mechanisms should be involved. Aromatic hydrocarbon receptor (AhR) activation increases cell division in human breast cancer cells, and CPF strongly activates it. Finally, the KIAA1363 enzyme, which is regulated by CPF, is overexpressed in cancer cells. Accordingly, we hypothesized that CPF or its metabolite chlorpyrifos-oxon (CPFO) could induce cell viability promotion in MCF-7 and MDA-MB-231 cell lines, through mechanisms related to ERα, AhR, and KIAA1363, after 24 h and 14 days treatment. Results show that, after acute and long-term treatment, CPF and CPFO alter differently KIAA1363, AhR, ER and CYP1A1 expression. In addition, they induced cell proliferation through ERα activation after 24 h exposure in MCF-7 cells and through KIAA1363 overexpression and AhR activation in MCF-7 and MDA-MB-231 cells after acute and long-term treatment. The results obtained in this work provide new information relative to the mechanisms involved in the CPF toxic effects that could lead to breast cancer disease.

2328 Development of a Strategy to Bridge Agrochemical Formulations Based on Historical Acute Toxicity Data and Formulation Composition

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To reduce the use of vertebrate testing, alternative approaches including bridging arguments can be submitted in lieu of studies. Historically, as part of an application package for a new agrocultural pesticide formulation, a defined battery of acute toxicity studies known as the "6-pack" was required. While the opportunity to bridge a new pesticide formulation from previously assessed formulations is attractive, the criteria that factor into a reliable bridging argument is not well defined. We assessed historical acute toxicity data to develop a step-wise approach in order to confidently and effectively bridge

across similar formulations. As a starting point we focused on acute oral toxicity data from formulations for which two or more versions of a formulation have been tested. We compared the oral LD_{50}, the EPA and GHS classifications, and the formulation composition of greater than 250 variants. Composition, study design and reported LD_{50s} were investigated to help establish reasons for similarities and differences. This gave us a broad overview of considerations for bridging rationales. The ultimate goal is to understand what composition changes do and do not lead to a change in the acute toxicological profile of agricultural pesticide formulations and provide confidence in bridging assessments.

2329 Environmental Impact of Underwater Released Exhaust Gas from Ships and Its Potential as Antifouling Strategy

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In an effort to reduce harmful emissions and increase energy efficiency, the application of Liquefied Natural Gas (LNG) as fuel for ship engines is being studied in combination with an underwater exhaust system to reduce the ship's water resistance. Besides inert nitrogen gas, hydrocarbons and nanoparticles, the exhaust gas consists 5-8% of CO_2, which may significantly increase CO_2 levels in the water around the ship. Apart from the assessment of the impact on the local environment, we investigated if underwater released exhaust gas could be used as anti-fouling strategy for ship hulls, as alternative to the traditional coatings that are often highly toxic to non-target marine organisms. Barnacles and biofilm were exposed for 25 days in seawater to a wide range of elevated CO_2 concentrations (pH 6.3 - pH 7.8). With decreasing pH from 7.8 to 6.3, the development of biofilm biomass was significantly reduced and a shift in taxonomic composition was observed. It clearly indicated that elevated CO_2 concentrations can slow down the formation of the initial biofilm, which could also have implications for the settlement of fouling invertebrates like barnacles and mussels. Barnacles tolerated acidic conditions up to pH 6.8. However, at the highest exposure condition (equivalent to pH = 6.3) feeding activity and growth were significantly inhibited and mortality increased. From these animals the adhesive power was reduced such that the water force of a relatively slow sailing speed of 12 km h^{-1} was sufficient to remove over 50% of the barnacles. Based on physiological features, it is expected that smaller animals, new larvae and fouling molluscs will be more sensitive. Our results findings suggest that CO_2 released along a ship's hull could prevalence of biofilm development and even combatting attached barnacles, although more research is needed to fully understand the mechanisms involved and the potential for practical application.

2330 Effects of *Cymbopogon nardus* (L.) Essential Oil on the Life Cycle and Midgut of *Ceraeochrysa claveri*

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The essential oil (EO) from *Cymbopogon nardus* is known as citronella oil, and it has been used as insect repellent, household fumigant, and fragrance agent in food commodities, soaps and cosmetics. The aim of this study was to characterize this EO by GC/MS, and evaluate its effects on the life cycle and midgut morphology of *Ceraeochrysa claveri* fed on eggs of *Diatraea saccharalis* (Lepidoptera: Crambidae). Fresh eggs of *D. saccharalis* were immersed in solutions of the EO (1-100 ppm in methanol, 5 s) or solvent (control) and dried at room temperature (30 min). Newly hatched larvae were randomly selected, divided into four experimental groups (n=15), and placed in polyethylene cups until the adults emerged. These were fed with an artificial diet (1:1 honey/yeast solution). One day after, insects were used to obtain their midgut. Larval and pupal stage duration, the emergence time of the insect, as well as the frequency of malformed organisms were recorded. Major constituents of the EO were citronellal (32.8%), geraniol (21.9%), citronellol (21.5%), germacrene D (3.3%), citronellyl acetate (2.9%) and geranyl acetate (2.1%). Insect mortality followed an EO concentration-response relationship. There was a significant change ($p<0.05$) in the duration of the third instar and prepupa stages, as well as in the total life cycle of insects exposed to the EO when compared to the control group. Observed alterations in the life cycle of *C. claveri* exposed to the EO included prepupa with no cocoon formation, death pupa inside cocoon, and malformed adults. Serious injuries on the midgut epithelium of exposed adults were observed, including detachment of columnar cells, leaving only swollen regenerative cells fixed on the basement membrane, and the formation of epithelial folds. In summary, these data suggest the EO of *C. nardus* has adverse effects on the life cycle of *C. claveri* and causes damage to its intestine. *COLCIENCIAS 567-2012; FP 44842-212-2018; Unicartagena, Resolution 01153, 2016.*

2331 Effects of Some Water Quality Parameters on Histological and Hematological Profiles of Two Important Fish Species from Ogun Waterside Lagoon System, Nigeria

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Seasonal variations in physical and chemical parameters of Makun-Omi lagoon system and their effects on the health status of *Gymnarchus niloticus* and *Coptodon zillii* were determined using histopathological and haematological profiles. Water and fish samples were randomly collected during the dry and wet seasons and were analysed following standard procedures. Data collected were subjected to Analysis of Variance and correlation statistical analysis using SPSS (version 20). Duncan Multiple Range Test was used to separate the means at $p<0.05$. A significant difference ($p<0.05$) existed between seasons in the physical and chemical parameters of water samples. Recorded values of the water quality parameters for the dry and wet seasons were: Temperature 30.60±0.46 and 28.57±0.23 °C, pH 6.53±0.88 and 6.43±0.67, Dissolved Oxygen (DO) 2.80±0.12 and 3.03±0.88 mg/l, Electrical Conductivity 180.00±2.89 and 244.33±5.81 µS/cm, Salinity 1.00±0.00 and 0.93±0.07 ‰, Total Alkalinity 12.00±0.29 and 8.83±0.20 mg/l, Total Hardness 56.67±0.67 and 52.00±1.16 mg/l, Total Dissolved Solids 84.33±0.33 and 131.67±2.67 mg/l, Phosphate 0.001±0.003 and 0.002±0.003 mg/l, and Nitrate 7.70±0.12 and 5.97±0.09 mg/l respectively. The selected heavy metals concentrations (Cadmium, Chromium, Cobalt, Lead and Nickel) were below detection limit in the water samples during the wet season, compared to their varying levels of detection during the dry season. Nickel (Ni) concentration in water was 0.264±0.00 mg/l; Cobalt was below detection limit in the tissues of experimental species in both seasons, while concentrations of other metals varied with season and in the tissues. The gills of *C. zillii* and liver of *G. niloticus* had concentrations of 0.190±0.03 mg/kg and 0.136±0.01 mg/kg respectively. Haematological parameters of *G. niloticus* showed significant difference ($p<0.05$) between seasons. Packed cell volume (21.40±1.30 and 22.40±1.08%), Haemoglobin (7.40±0.41 and 7.38±0.33 g/dl) and Red blood cell ($1.89x10^{12}$±0.12 and $2.03x10^{12}$±0.11/L) for dry and wet seasons respectively. However, there were relatively high values of White blood cell, heterophils and lymphocytes ($10.92x10^{12}$±0.11/L, 43.80±1.96% and 58.80±1.88%, respectively) in the fish. A strong positive relationship existed between water and haematological parameters with r values of 0.77 and 0.75 for dry and wet seasons respectively ($p<0.05$). Histological results revealed moderate to acute alterations in the tissues of the examined fish species. The gills of both fish species had more alterations in the dry season as against the wet season, while the liver of *G. niloticus* showed more alterations in the wet than the dry season. Haematological and histopathological observations showed varying impacts on the blood and tissues of the fish species, given the extreme values of DO, nitrate and Ni concentrations in water. The study concluded that environmental monitoring of the water body is recommended in order to control the effects of water pollution which ultimately affects the health status of the fish species.

2332 Toxicity Assessment of Sediments from a River Watershed Impacted by Open-Pit Coal Mining in Colombia

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Coal mining is one of the most important economic activities at the Northern Colombia. However, mineral extractions are carried out near rivers that provide ecosystem services to riverside populations. Cesar River receives discharges from open-pit coal mines, as well as from other anthropogenic sources. The aim of this work was to assess the toxicity profile of the sediments from the Cesar River. Bottom sediment samples were collected from 12 points along the course of the river, including tributaries and a Ramsar site, the Zapatosa Marsh. Samples were freeze-dried, and the <75 µm fraction was employed to obtain 1:3 ratio aqueous extracts (K-medium). The biological effects of the sediments were evaluated using *Caenorhabditis elegans*. Wild-type Bristol N2 nematodes were exposed to the extracts, registering mortality, locomotion and growth as end points. Transcriptional effects associated with various toxicity mechanisms were assessed using GFP-related transgenic strains (mtl-2, sod-4 and gst-1). Trace elements were quantified employing ICP-MS, and mercury (Hg) was measured using a direct Hg analyzer. Sediment

extract-induced lethality was low (1.5-6.4%); however, nematode growth and locomotion decreased along the river, showing inhibition rates up to 23.3 and 35.4%, respectively. Extracts induced over expression of all tested genes, with greater values for sites receiving domestic sewage and mining outputs. Some trace metals enriched along the course of the river, especially Hg and V. Cobalt was positively associated with the mRNA expression of metallothioneins and gst-1. Greatest biological impacts from sediments extracts were observed downstream the coal-mining area and in Zapatosa Marsh. These data suggest coal mining areas should be closely monitored for trace-element release and their impact on biota. The Colombian government should implement laws, and programs aimed to protect key ecosystems from mining activities, as a commitment to sustainable development goals. *UniCartagena 096/2018.*

2333 Genotoxicity, Histopathological Examination, and Heavy Metals Assessment of *Chrysichthys nigrodigitatus* at Makoko Fish Landing Site, Lagos, Nigeria

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A total of sixty (60) Samples of *Chrysichthys nigrodigitatus* were collected for four months from the Makoko area of the Lagos lagoon. Water, sediment, fish liver and blood samples were collected from three selected stations in Makoko fishing area and assessed. Physicochemistry, heavy metals (Zn, Pb, Cu and Fe), liver pathology and erythrocytes abnormalities were assessed. Total of sixty (60) (five from each station at each sampling period) were collected. Heavy metals in water and sediment media were analyzed using atomic absorption spectrophotometer. Six nuclear abnormalities in erythrocytes were considered; Micronucleated, Binucleated, Blebbed, Notched, Dumbbell and Irregular. Hepatopathological Alteration Index (HAI) and Hepatosomatic Index were also estimated. All the measured variables were still within WHO limits (1989) with station 2 having the highest BOD and DO concentrations; Mean±SD, 6.69±0.32 mg/l and Mean±SD, 6.11±0.15 mg/l respectively. The surface water in the three stations had copper, iron and zinc but very low lead. These metals were within WHO limits except for zinc in August, 2018, Mean±SD, 3.19±5.19 (mg/l). There were higher concentrations of the metals (Fe, Cu, Zn and Pb) in sediment as compared to surface water. These concentrations were below WHO limits. There were high frequencies of nuclear abnormalities in erythrocytes of *Chrysichthys nigrodigitatus* collected each month at the sampled areas. August had the highest frequency with total sum frequencies of abnormalities in cells as 110 with sum average frequency of 21.8 and the trend, August>October>July>September. The distribution of the frequencies of abnormalities across the months were significant at 0.05 level, Chi-Square, X^2 (3) = 12.613, $p<0.05$. The frequency of Binucleated cell was the highest nuclear abnormalities with total frequency of 118 and average frequency of 23.4 and the trend Binucleated >Multinucleated >Notched >Blebbed>Irregular> Dumbell. Lead and copper concentrations in the sediment correlated negatively with the frequency of Binucleated cells in *Chrysichthys nigrodigitatus* ($r = -0.705$, $p<0.05$ and $r = -0.630$, $p<0.05$ respectively). More so, sediment zinc ion correlates with sediment copper ion ($r = 0.628$, $p<0.05$). August had the highest surface water metal load, suspected to be an industrial discharge, with zinc concentration above WHO limit. There was relationship in certain nuclear abnormities and histological alterations in the fish and certain heavy metals in the surface water and sediment media due to pollution.

2334 Wildlife Toxicity Assessment for 2,4-Dinitroanisole (DNAN)

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2,4-Dinitranisole (DNAN), also known as 1-metoxy-2,4-dinitrobenzene is used in explosive mixtures. Currently, it is being investigated as a component in replacements of other conventional energetic Military munitions. It is used as a component of insensitive munition explosive (IMX) formulations. For example, IMX-101, which is comprised of nitrotriazolone (NTO); DNAN; and nitroguanidine is a comparatively less sensitive explosive formulation than many conventional formulations. When fielded, DNAN may be detected in soil, surface water, and potentially in animal and plants at manufacturing facilities and where training operations occur. We have reviewed the acute and sub-chronic toxicity data of DNAN in mammalian, avian, reptilian and amphibian species. Despite limited wildlife exposure data in animal studies, we have attempted to derive points of departure (PODs) from dose-response data reviewed from target organ-specific studies. US Army Public Health Center Technical Guide 254 - Standard Practice for Wildlife Toxicity Reference Values (TRVs), guided our development of class-specific TRVs. Recently developed data are available for mammals, avian, and some amphibian species. The data relevant to reptiles are absent. Tentatively derived PODs (mg/kg-day) were derived from a sub-chronic study conducted in rats, and Benchmark dose (BMD) values were derived from studies of exposure to DNAN by ingestion in mammals. A BMD or ED10 of 1.78 mg/kg-day, and a lower-bound BMDL10 or LED10 value of 0.93 mg/kg-day was determined for the class Mammalia. For inhalation exposures, a LOAEL-based TRV of 165 mg/m^3 and a NOAEL-based TRV of 4.1 mg/m^3 for the class Mammalia were determined. For ingestion TRVs for the class Aves, a LOAEL-based TRV of 7.5 mg/kg and a NOAEL-based TRV of 1.5 mg/kg were derived following oral exposure to DNAN. For amphibian species, a NOAEC TRV of 0.24 mg/L and a LOAEC TRV of 2.4 mg/L were derived following a 28-day DNAN exposure. Tentative TRVs were based on data from the most sensitive species available, and are intended to be protective of the entire class of wildlife species with broad utility in ecological risk assessment

2335 Regioselective Effects of OxyPAHs on Red Blood Cell Concentrations in Japanese Medaka Embryos

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Previous studies in zebrafish have demonstrated that exposure to oxygenated polyaromatic hydrocarbons (oxyPAHs) at critical developmental time points can impair red blood cell (RBC) concentrations in a regioselective manner, with 2-hydroxychrysene being more potent than 6-hydroxychrysene. Japanese medaka embryos were exposed to 2 or 6-hydroxychrysene (0.5, 2, or 5μM) or 70μM phenanthrene (a positive control) from 4h post-fertilization (hpf) to 7d post-fertilization (dpf). Following exposure, RBC concentrations were quantified by staining fixed embryos with *o*-dianisidine (a hemoglobin-specific dye), imaging stained embryos using brightfield microscopy, and analyzing images within ImageJ. Exposure of embryos to all three concentrations of 2-hydroxychrysene resulted in a significant decrease in hemoglobin concentrations compared to vehicle controls, while there were no effects observed following exposure to 6-hydroxychrysene at all tested concentrations. Embryonic uptake rates for 2 and 6-hydroxychrysene were also evaluated by determining body burden at multiple timepoints of exposure by liquid-liquid extraction and LC-fluorescence analysis and there was no significant difference between the rate of uptake for both compounds. Medaka embryos were exposed to 2-hydroxychrysene at the beginning of four different developmental stages (13-15hpf, 38-41hpf, 82-95hpf, 95-101hpf) to identify a window of sensitivity for toxicity. Unlike initiation of exposure at 4hpf, none of these exposures resulted in the no-blood phenotype nor mortality. However, when Medaka embryos were exposed to 2-hydroxychrysene for 24, 48, 72, and 96 hours beginning at 4hpf, the 48, 72, and 96h exposure durations resulted in significant changes in phenotype and survival. These data indicate that regioselective toxicity is not due to differences in uptake and indicate other mechanisms may be involved. *This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER2).*

2336 Intergenerational Toxicity of Nonylphenol Ethoxylate (NP-9) in *Caenorhabditis elegans*

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The ethoxylated isomers of nonylphenol (NPEs, NP-9) are one of the main active ingredients of many nonionic surfactants used in herbicides, cosmetics, paints, plastics, disinfectants and detergents. These chemicals and their metabolites are commonly found in environmental matrices. The objective of this work was to evaluate the intergenerational toxicity of NP-9 in *Caenorhabditis elegans*. The survival rate and locomotion were investigated in the larval stage L4, while length, width and longevity were assessed using the L1 stage of the wild strain N2. Green fluorescent protein (GFP) transgenic strains were employed to estimate changes in relative gene expression. RT-qPCR was utilized to measure mRNA expression for different genes associated with neurotoxicity (unc-30, unc-25, unc-49, dop-3, dat-1, mgl-1, eat-4, glt-3 and glt-6) and oxidative stress (mtl-1 and mtl-2). Data were obtained from exposed parent worms (F0) and the first non-exposed generation (F1). Lethality of the nematode was concentration-dependent, with 24 h-LC50 values of 3.2 and 2.0 mM for F0 and F1, respectively. Non-lethal concentrations of NP-9 reduced locomotion and longevity, whereas non-monotonic concentration-response curves were observed for body length and width in both generations. The gene expression profile in F0 was different from that registered in F1, although the expression of sod-4, hsp-70, gpx-6 and mtl-2 increased with the surfactant concentration. In F0, NP-9 induced non-monotonic responses for genes related to oxidative stress, GABA, dopamine and glutamate; whereas in F1, most genes displayed classical concentration-response curves. In summary, NP-9 induced intergenerational responses in *C. elegans* through mech-

anisms involving ROS and alterations of the GABA, glutamate and dopamine pathways. *Colciencias-UniCartagena, 727, 2015. Vice-Rectory for Research. 2017-2019.*

2337 Establishment of Yeast-Based Reporter Gene Assay Using Methoprene-Tolerant of European Honey Bee, *Apis mellifera*

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Juvenile hormones (JHs) are one of the hormones that control growth and development of arthropods. In social insects including honey bees, JHs influence the caste differentiation and regulation of division of labor. Honey bees are important organisms responsible for pollination of various agricultural crops. Recently, colony collapse disorder, in which honey bee colonies lose worker bees, are concerned as serious problem for ecosystem. *Krüppel homolog-1* (*Kr-h1*) is target gene of JH receptor Mehoprene-tolerant (Met) in insect. In *A. mellifera*, a correlation between behavioral development of worker bees and expression of *Kr-h1* was previously reported. Therefore, chemicals with JH-like activity used for pest control may affect the endocrine system of bees, leading the decrease of population. In this study, we established yeast strains expressing Met of *A. mellifera* to detect JH-like substances in the environment. *A. mellifera* Met, *A. mellifera* steroid receptor coactivator (SRC), and L*acZ* reporter plasmid carrying putative JH response elements with E-box (CACGTG) located in upstream region of *A. mellifera Kr-h1* were constructed. Three plasmids were transformed into *Saccharomyces cerevisiae* W303 using standard lithium acetate method. Established reporter gene assay showed dose-dependent responses to an endogenous ligand JHIII via E-box-containing sequence of *Kr-h1* gene. In larvae of *A. mellifera*, mRNA levels of *Kr-h1* were significantly increased after JHIII treatment. These results indicate that *Kr-h1* is a target gene of Met in *A. mellifera* as well as other insects, and this assay is useful as an easy, rapid and cost-effective primary screening tool to detect JH-like activity for *A. mellifella* Met. *First and second authors contributed equally to the work.*

2338 Whole-Transcriptome Sequencing of Lake Trout Epidermal Mucus as an Exposure Assessment Tool following Low-Level Additions of Diluted Bitumen in a Boreal Lake

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To prevent further disruption of wild populations, development of noninvasive sampling methods has become increasingly important when studying toxicological effects of environmental pollutants on aquatic organisms. In fish, analysis of epidermal mucus is a promising route for development of noninvasive sampling methods. In a previous study in our lab, whole-transcriptome sequencing of mahi-mahi (*Coryphaena hippurus*) epidermal mucus following slick oil exposure revealed that transcriptional responses in the mucus paralleled those in whole-embryo homogenates. Furthermore, hierarchical clustering of samples created separate clades for each treatment group, suggesting that sequencing of mucus is able to robustly capture transcriptional profile differences among individuals. To further assess the efficacy of mucus as an exposure assessment tool, RNA sequencing was performed on mucus collected from lake trout (*Salvelinus namaycush*) residing in a boreal lake that received low-level additions of diluted bitumen (dilbit), a form of crude oil, at the Experimental Lakes Area. Sequencing results from exposed fish will be compared to sequencing results from fish prior to dilbit exposure as well as from fish from a nearby non-exposed lake. Through sequencing, we will identify differentially expressed genes in dilbit-exposed individuals which will provide insight on biological pathways and functions that are altered following dilbit exposure. Along with this, we will assess whether mucus transcriptional profiles are able to robustly distinguish exposed from non-exposed fish. Overall, results of mucus sequencing will provide further insight on the efficacy of mucus as a nonlethal exposure assessment tool in a whole-ecosystem setting. *This research was made possible by a grant from The Gulf of Mexico Research Initiative. Grant No: SA-1520; Name: Relationship of Effects of Cardiac Outcomes in fish for Validation of Ecological Risk (RECOVER2).*

2339 Embryonic Development in Fish Is Hindered by Exposure to Environmentally Relevant Concentrations of Glyphosate

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Glyphosate-based herbicide use is increasing yearly to keep up with the growing demand of the agriculture world. Although glyphosate-based herbicides target the enzymatic pathway in plants, the effects on endocrine systems of vertebrate organisms, particularly fish, are widely unknown. The present study examined the effects of glyphosate exposure on embryo development and the thyroid system of the Japanese Medaka fish (*Oryzias latipes*). The Hd-rR medaka embryos were exposed to Roundup containing 0.05, 0.5, 5, 10, and 20 mg/L glyphosate (glyphosate acid equivalent) from the 8 hours post-fertilization stage through 14 days post-fertilization stage. Phenotypes observed include delayed hatching, increased developmental deformities, growth, and embryo mortality. The lowest concentration (0.05 mg/L) and the highest concentration (20 mg/L) of glyphosate induced similar phenotypes in embryos and juveniles. Gene expression analysis revealed a significant decrease in acetylcholine esterase (*ache*) mRNA levels and thyroid hormone receptor alpha (*thra*) mRNA levels in juveniles exposed to 0.05 mg/L and 20 mg/L glyphosate. Quantification of epigenetic alterations of glyphosate is currently in progress. The present results demonstrate that glyphosate via exposure to Roundup affects early development of medaka in a non-monotonic dose response manner and that environmentally relevant concentrations of Roundup can be toxic to fish embryos and juveniles. *Supported by Biology Department, University of North Carolina Greensboro.*

2340 Determination of Pharmaceuticals in Water and Biofilm Samples from the Hudson River

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Pharmaceuticals are poorly degraded during conventional wastewater treatment, and may persist and accumulate in the receiving environment (biofilm). Despite the presence of a wide suite of pharmaceuticals in the Hudson River water, we are aware of no published studies on the presence of pharmaceuticals on Hudson River biota. The primary objective of this study was to develop a qualitative analytical method in river water and a quantitative analytical method in biofilm samples to detect 12 pharmaceuticals of high potential for environmental harm. The second objective was to investigate the pharmaceuticals' presence in these types of samples, collected from six locations along the Hudson River at three different time points throughout 2018 (n=18). These locations were chosen due to the proximity of a wastewater treatment plant and combined sewer overflows locations (n=8). One-hundred mL water samples were acidified and filtered. Biofilm samples (0.25 g) were homogenized via bead mill in 8 mL methanol. Both water and biofilm samples were further purified by mixed mode solid phase extraction. Samples were analyzed by liquid chromatography tandem mass spectrometry, utilizing positive electrospray ionization in multiple reaction monitoring mode. All compounds were analyzed simultaneously by reversed-phase in gradient mode with 0.1% formic acid in water and in acetonitrile as mobile phases. Biofilm quantitative method validation included linearity (1-100 ng/g), limit of detection (0.5 ng/g), imprecision (<20%), accuracy (80-120%), matrix effect and extraction efficiency. Water qualitative method achieved cut-offs of 10, 50 or 100 ng/L, depending on the compound. Two biofilm and 18 river water samples collected from six locations along the Hudson River tested positive. Biofilm samples were positive for sulfamethoxazole (n=1, 6 ng/g) and oxycodone (n=1, 0.4 ng/g). River water samples tested presumptively positive for atenolol (n=18), metoprolol (n=18), fluoxetine (n=1) and alprazolam (n=2). This study developed and validated sensitive and specific methods for the detection of 12 pharmaceuticals in biofilm and river water samples. Two pharmaceuticals, oxycodone and sulfamethoxazole, were detected with concentrations up to 6 ng/g in the biofilm samples from the Hudson River, and 4 pharmaceuticals, atenolol, metoprolol, fluoxetine and alprazolam were detected in river water samples.

2341 Control of Burrowing Shrimp: Modes of Action and Efficacy

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For 70 years, growers of Pacific oysters (*Crassostrea gigas*) in Washington State have found it necessary to control burrowing shrimp (*Neotropea californiensis*) on intertidal shellfish beds as they destabilize sediments resulting in poor survival and low yields. The carbamate, carbaryl (an AChE inhibitor) was used

to control shrimp until 2014 following litigation over non-target effects. The neonicotinoid, imidacloprid (IMI) was identified as an alternative, but the State has not approved its use citing the potential for non-target effects and growing concerns over the use of neonicotiniods. An outcry, primarily from chefs in Seattle and local shellfish buyers, over the use of the neurotoxin on beds from which oysters are harvested, coupled with poor efficacy, contributed to the controversy. As a result, Pacific County has declared an "Economic Emergency". For more than a decade, undergraduates at the University of Washington have conducted research on the toxicity of IMI to adult and juvenile shrimp as well as alternatives: emamectin benzoate (EB) and un-iodized table salt (TS). Studies in seawater (SW) indicate shrimp are extremely sensitive to IMI with 1-h EC50's (paralysis) in SW of 45 ppb for juveniles and 116 ppb for adults. However, lethal exposures exceed 12,000 and 1 mil ppb, respectively; exposures unlikely to be realized in the field at the maximum allowable application rate. Mortality of adults in sediment results from paralysis leading to burrow collapse and asphyxiation, but only if the paralysis lasts 54-78 h (i.e., IMI exposure dependent). Nicotinic ACh receptors are primarily located in the gastric mill (stomach) of shrimp, likely explaining the poor observed efficacy. In contrast, EB targets the primary neurophysiology of shrimp (glutamate/GABA) and has a US marine registration (SLICE®) as a feed additive for the control of sea lice (*Caligus* and *Lepeophtheirus* spp.) on farmed salmon. Exposures of adult and juvenile shrimp to EB (as Proclaim®) in SW yielded 96-h LC50s of >100 ppb and 18 ppb, respectively, with the former approaching the 96-h LC50 for juvenile salmonids, non-target species of concern. More promising are recent studies with TS. Adult and juvenile shrimp are very sensitive to acute changes in salinity with mortality occurring rapidly within 6 h when exposed to 2-3x ambient salinity. Studies in 2020 will focus on TS and juvenile shrimp within native sediment as they occupy the upper 10-15 cm, as well as juvenile Dungeness crab, a non-target species of concern.

PS 2342 Benthic Ecotoxicological and Seafood Risk Assessment in Hangzhou Bay, China

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The Hangzhou bay region has the fastest growing economic development and is one of the most contaminated coastal region of China. Recent research reveals high levels of toxic compounds in the sediments in this region. This implies many potential adverse consequences for the benthic ecosystem health and services it can provide, benthic eco-toxicity, seafood safety, and whole benthic ecosystem stability. This study aims to study the eco-toxicological effects in the field, apply chemical analysis and bioassays for monitoring the toxic potency of the sediment and to assess the toxicological risks for seafood originating from this region. The study firstly collected and analyzed historic monitoring data about marine sediment quality and benthic animals' species variety. Results revealed the key benthic pollutants are arsenic, copper, chromium and cadmium. More than 60% of benthic species are disappeared in last 20 years. Crustacean group animals are mostly survived compare to other species and have a big concern for contamination accumulation in the seafood crustacean species like swimming crabs. The second section of the study applied *in vitro* bioassays to quantify genotoxicity potencies of toxic compounds in marine sediments and benthic animals. The bioassay employs a multiplex of four reporter strains (key proteins) covering seven recognized DNA damage repair pathways. Via GFP fusion proteins this genomics assay enables quantification of the potency to activate multiple DNA damage mechanisms by compounds extracted from sediments and from benthic animals. Chemical analysis was performed for calibration of the bioassay. Results illustrated swimming crab samples showed a very high response to umuD and lexA genes and it can strongly relate to genotoxicity effects. Late chemical analysis proves crabs' samples showed gene response does contain high PAHs levels compare to the safety level. In the third section of this research, sediment and swimming crabs are assessed seafood and ecological risk. The assessment of sediment showed moderate ecological risk mainly caused by multiple heavy metals. Food risk assessment illustrated Cadmium and Naphthalene from swimming crab has very high food risk to humans in the acute and chronic period. All in all, the study found Hangzhou bay the region does have many ecological and seafood concerns in the benthic ecosystem. Arsenic, chromium, and cadmium are exceeding safety levels and more than half benthic animal species are disappearing in the last twenty years. Genotoxicity is found in seafood samples and moderate risk is assessed from sediment. Meanwhile, *in vitro* bioassays and risk assessment were proved can be alternative tools in the region to find out all the hidden hazards and support quantitative risk study.

PS 2343 Changes in Embryonic Zebrafish Cardiac Gene Expression following Crude Oil Exposure

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Crude oil and its degradation byproducts are known to have cardiotoxic effects on early life-stage fish including commercial species such as tuna and salmon. Exposed fish often exhibit pericardial edema and impaired heart function at very low exposure concentrations. This study examined the timed induction of several genes in response to crude oil exposure, including cytochrome P450 (*cyp1a*) and bone morphogenic protein 10 (*bmp10)* in embryonic zebrafish (*Danio rerio*), a model organism. *Cyp1a* is a widely studied gene involved in the metabolism of xenobiotic (foreign) substances. *Bmp10* is a cardiac gene involved in Ca^{2+} signaling, and is a likely contributor to oil-induced cardiac abnormalities. However, the timing of cardiac gene induction after crude oil exposure is unknown in both zebrafish and commercially important fish species. In this study, zebrafish were continuously exposed to a crude oil water-associated fraction (WAF) starting at 24 hours post fertilization (hpf) with four replicates per treatment including water-only controls. Twenty embryos were sampled per replicate at seven timepoints between 24hpf and 48hpf. RNA was extracted from each sample and converted to cDNA. Using a comparative Ct method, expression changes in genes of interest were measured using real time quantitative polymerase chain reaction (rt-qPCR), and analyzed using various statistical tests in R. Statistically significant upregulation of calcium signaling genes support a decrease in fitness of early life-stage zebrafish after exposure to low concentrations of crude oil. Likewise, this study has direct implications to the health and viability of commercially important fish exposed to oil spills in marine environments.

PS 2344 Route of Exposure Influences Pesticide Body Burden and the Hepatic Metabolome in Post-Metamorphic Leopard Frogs

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Pesticides are being applied at a greater extent than in the past due in part to availability of advanced formulations and/or new marketed varieties. Once pesticides enter the ecosystem, many environmental factors can influence their residence time. These interactions can result in processes such as translocation, environmental degradation, and metabolic activation upon exposure to target and non-target species. Most anurans start off their life cycle in aquatic environments and then transition into terrestrial habitats. Their time in the aquatic environment is generally short; however, many important developmental stages occur in this environment. Post-metamorphosis, most species spend many years on land but migrate back to the aquatic environment for breeding. Due to the importance of both the aquatic and terrestrial environments to the life stages of amphibians, we investigated how the route of exposure (i.e. uptake from contaminated soils vs. uptake from contaminated surface water) influences pesticide body burden (for bifenthrin (BIF), chlorpyrifos (CPF), glyphosate (GLY), and trifloxystrobin (TFS)) and the hepatic metabolome in adult leopard frogs. For water exposures, 50 mL of pesticide contaminated water was added to a Pyrex® bowl and for soil exposures a thin layer of soil was added to the bottom of a bowl, followed by the pesticide application using an aerosolized spray bottle, final exposure concentrations were 1 µg/mL. Body burden concentrations for amphibians exposed in water were significantly higher (ANOVA $p < 0.001$) compared to amphibians exposed to contaminated soil across all pesticides. Furthermore, the metabolite TFS acid was also significantly higher in water than in soil (ANOVA $p < 0.05$). The pesticide with the highest concentration in frogs was CPF in the water exposures while the lowest pesticide body burdens were determined for BIF and TFS in the contaminated soil exposures. Ultimately, this research will help fill regulatory data gaps, aid in the creation of more accurate dermal exposure and uptake models for amphibians and inform risk assessment efforts for these and other non-target species.

2345 Screening of Metals in Andean Condor Feathers: Possible Influence of Vulcano Eruption

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Northern Patagonia is a sparsely populated area, where volcanic eruptions represent an important source of metals (heavy metals and metalloids) pollution. The great eruption of the Puyehue-Cordón Caulle volcanic complex (PCCVC) (Chile) in 2011 reached the surroundings of Bariloche (Argentina) (> 100 km). The effects of ash exposure on wildlife and humans have been sparsely studied and biomonitoring studies with higher species (e.g. Andean condor; *Vultur gryphus*) have never been conducted. The main objectives of our study were the metal screening and evaluate a possible relation between the levels of metals in the Andean condor population and the volcanic activity of the area. We investigated the effects of the eruption of the PCCVC in 2011, using samples of molt primary feathers of the Andean condor, collected in nine roosts around Bariloche, Argentina (maximum distance 85 km). The information we currently have made us hypothesize that the molt of the primary feathers of the Andean condor has duration of 6 years. We carried out sampling before (2007, 2009) and after (2017) the volcanic eruption. The feathers sampled in 2017 should have been developed in 2011-2012, reflecting the environmental situation of the period immediately following the eruption of the PCCVC. For the first time we have screened metals in 48 molted primary feathers of Andean condor, showing the levels of 9 metals and metalloids (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg). Si, Zn, As, Cd, showed higher levels in the feathers sampled after the eruption. This fact seem to confirm the volcanic contamination (Si represent 70% of the PCCVC ashes). The levels of Cr and Pb (although apparently not related to the volcanic eruption) in some samples are compatible with potential adverse effects in living beings. The screening results represent an important database (first for this species) that can be used in in future studies for comparative purposes.

2346 Proteomic Analysis of Anatoxin-A (±) in Zebrafish (*Danio rerio*)

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Cyanobacteria have a broad geographical distribution and can be found in both freshwater and marine environments. Multiplying quickly in surface waters, they form blooms when favorable conditions prevail with > 2000 species occurring globally in aquatic habitats. It is estimated that of blooms which form rapidly, 25-70 % may be toxic. Anatoxin-A and its analogues are potent neurotoxins produced by several genera of cyanobacteria. Examination of the literature results in a failure of toxicity studies to differentiate between enantiomerically or chemically pure anatoxin-a. A recent study summarized that of 33 studies examined for toxicity data, 18 reported on the use of the racemic mixture. However, little is known about the toxicological impact of this mixture at the protein level. Using nano LC-MS/MS protocol through a 2D-nanoAcquity UPLC with dilution coupled to a quadrupole time-of-flight (Q-Tof) Synapt G2 MS, protein differences were characterized in larval Zebrafish following a 96 h exposure at environmentally relevant concentrations. Altered proteins were predominantly down-regulated with respect to control samples (~129-278, 5 % FDR). Altered proteins identified had varying functions in carbohydrate metabolism and energy production, stress response, ATP synthesis and cellular development. The study provides valuable insights into how Anatoxin-a exerts its toxicity in fish.

2347 Oxidative Degradation of Waterborne Microcystins: Studies of Ozone-Mediated Degradation of Methyl 2-Acetamidoacrylate to Model Oxidative Inactivation of Microcystin-LR

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Water resources for humans and farm animals are becoming increasingly scarce in recent years, in part, due to algal blooms. Fresh water algal blooms involving cyanobacterial species (e.g., *Anabaena and Microcystis*) result in the formation of microcystins (MCs), the most toxic one being microcystin-LR (MC-LR). The unusually high toxicity of MCs is often attributed to the presence of N-methyldehydroalanine (Mdha) moiety present as part of oligopeptide ring system. Mdha serves as the principal electrophilic center in reactions of MCs with protein phosphatases resulting in their irreversible inactivation. In view of its potential relevance to oxidative inactivation of MC-LR and other MCs present in potable water, in the present study, we have examined reactions of ozone (O_3) with methyl 2-acetamidoacrylate (M2AA), the latter being used as a model for Mdha moiety in MCs. It was found that the M2AA-ozone reactions performed at pH 2.0 and 7.0 have nearly identical changes in the absorption spectrum as those performed in deionized water. The pH-independent nature of the O_3-mediated oxidation and the presence of a single isosbestic point for reactions of M2AA with O_3 suggest that the reaction is a simple process wherein the initial step involves the formation of a primary ozonide (1,2,3-trioxolane) between O_3 and the olefinic (C=C) group of M2AA. From that stage, there is normal breakdown of the primary ozonide to carbonyl oxide and the aldehyde (or ketone) primary/secondary products. The reversed-phase HPLC analysis of the ozonation mixtures showed little or no evidence for the formation of secondary ozonide(s) of M2AA resulting from the retroaddition of carbonyl oxide and the adehyde product. The O_3-mediated oxidation appears to remove M2AA by >99% and results in the formation of aldehyde and ketone products, viz., methyl 2-acetamido-2-oxoacetate (analyzed by high resolution MS) and formaldehyde, i.e., in addition to H_2O_2. The formation of methyl 2-acetamido-2-oxoacetate and H_2O_2 were found to be stoichiometric with the yields of oxidation of M2AA. Based on the results presented, we believe several different oxidant systems consisting of either singly or in combinations of hypohalous acids, dichlor, trichlor, O_3/H_2O_2 and H_2O_2/UV, used during the pre- or post-treatment processes of potable and reclaimed water, can be studied similarly using M2AA to arrive at the most efficient method(s) of inactivation of MCs.

2348 *In Vitro* and *In Silico* Assessments of Dioxins for Polar Bear Aryl Hydrocarbon Receptor Transactivation Potency

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Some of polar bear (*Ursus maritimus*) populations accumulate dioxins and related compounds (DRCs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar dioxin-like polychlorinated biphenyls (DL-PCBs) over thousands times of human OECD provisional tolerable monthly intake (70pg TEQ/kg bw). The aryl hydrocarbon receptor (AHR) mediates most of toxicities of DRCs. To evaluate the sensitivity and responses to DRCs via AHR in polar bears, we assessed the transactivation potencies of DRCs through polar bear AHR (pbAHR) by *in vitro* and *in silico* approaches. We constructed an *in vitro* reporter gene assay system that pbAHR expression plasmid and a reporter plasmid containing CYP1A1 promoter were transfected in U2OS cells. The treatment with each of nine DRC congeners induced dose-dependent responses in the *in vitro* assay system. The pbAHR was as sensitive to DRCs as C3H/*lpr* mouse AHR which is known to be highly sensitive to DRCs. Comparison of pbAHR transactivation potencies indicated that 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF had high induction equivalency factors (IEFs). Considering the accumulation levels of DRCs in polar bears, PCB126 was predicted to be the most active to pbAHR. The *in vitro* transactivation potencies of ligands for pbAHR exhibited a significant relationship with *in silico* ligand docking energies in a pbAHR homology model constructed from the human HIF-2α template, 3H7W. The protein ligand interaction fingerprint (PLIF) analysis suggested that several amino acids, which are highly conserved among mammals, may be involved in the species-specific responses to DRCs via backbone interactions with neighboring amino acid residues, which are specific to pbAHR.

2349 Metabolomic Profiling to Inform Use of Surrogate Species in Ecological Risk Assessments

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The US Environmental Protection Agency (US EPA) routinely uses avian and fish toxicity data to set protective standards for amphibians in ecological risk assessments. However, this approach does not always adequately represent aquatic-dwelling and terrestrial-phase amphibian exposure data. While there are multiple amphibian families within the US EPA's ECOTOX database, a lack of concordance of standardized tests used to collect amphibian data limits the ability to estimate the ecological effects of pesticides in these species and affirm the use of surrogate species data. For instance, it is widely accepted

that early life stage tests for fish are typically sensitive enough to protect larval amphibians, but metamorphosis from tadpole to a terrestrial-phase adult relies on endocrine cues that are less prevalent in fish. These differences suggest that more robust approaches are needed in order to adequately elucidate the impacts of pesticide exposure in amphibians across critical life stages. Therefore, in the current study, the perturbations in the metabolomic response of larval zebrafish (*Danio rerio*), a surrogate species frequently used in ecotoxicological studies, are compared to those of African clawed frogs (*Xenopus laevis*) and southern toad (*Anaxyrus terrestris*) tadpoles following exposure to high-use pesticides. These species were exposed to pesticides including, but not limited to, atrazine, bifenthrin, chlorothalonil, metolachlor, tebuconazole, or trifluralin over a targeted range spanning reported values beneath those eliciting acute toxicity. For metabolomic profiling, liver tissues or whole organisms were liquid-liquid extracted, derivatized and analyzed by gas chromatography coupled with mass spectrometry. Following spectral alignment and preprocessing, multivariate analysis was utilized to identify dose-dependent changes in the metabolome for each species and each pesticide. Numerous biochemical pathways, such as fatty acid synthesis, amino acid metabolism and the citric acid cycle are frequently impacted by pesticide exposure. Ultimately, data gathered will help inform the applicability of the use of surrogate species in establishing the risk pesticide exposure poses to amphibians and potentially other non-target species.

PS 2350 Development of Knockout Zebrafish (*Danio rerio*) to Understand the Protective Role of Rho-Class Glutathione *S*-transferase in Chemical-Induced Oxidative Stress

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Glutathione *S*-transferases (GSTs) are major detoxification enzymes important in cellular protection by metabolizing endogenous and exogenous substrates such as chemotherapeutic agents, insecticides, herbicides, and by-products of oxidative stress. Most higher organisms express a number of GST isoenzymes, including *alpha*, *mu*, *pi*, *omega*, *theta*, and *zeta*, but an additional GST class (GST rho, *gstr*), is found only in fish and other aquatic organisms. In this study, we developed zebrafish (*Danio rerio*) *gstr* knockout lines, then conducted sublethal and lethal dose toxicity studies using tert-butylhydroperoxide (TBHP) to determine the toxicological relevance of the *gstr* isoform in chemical-induced oxidative stress. Mutant zebrafish *gstr* knockout lines were developed through genome editing using CRISPR/cas9 targeting the coding sequence of zebrafish *gstr*. Wild-type and knockout zebrafish were exposed to TBHP at 96 hpf (hours post fertilization), and percent survival was documented every 24 hours. Fluorescence microscopy of acridine orange staining was used to image apoptotic cells in larval zebrafish 72 hours post toxicant exposure. Following a 72 hour exposure, *gstr* knockout zebrafish exhibited a distinctly steeper survival curve compared to that of wild-type zebrafish 168 hpf at 110 mg/L TBHP following 72 hours of exposure. Furthermore, following exposure to a sublethal concentration (70 mg/L) of TBHP, 168 hpf knockout zebrafish expressed increased presence of apoptotic cells in the body cavity, and were more likely to develop physical deformities such as spinal curvature and uninflated swim bladders. Ongoing studies are examining the effects of *gstr* of other of environmental chemicals that induce oxidative stress. Our studies indicate that the rho class GST isoform unique to aquatic organisms is likely a major antioxidant enzyme that mediates protection against oxidative stress. *Supported by NIEHS Superfund ES04696.*

PS 2351 Generation and Characterization of Monoclonal Antibodies against Killifish, *Fundulus heteroclitus*, AHR1a, AHR1b, AHR2a, AHR2b, and AHRR

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The AHR is a member of the basic helix-loop-helix (bHLH-PAS) superfamily of proteins and functions as a ligand-activated transcription factor for multiple responsive gene products. Most notable of these include phase I and II drug-metabolizing enzymes and transporters. Other gene products include proteins associated with cell cycle arrest, such as p21, p57, and p27. The AHR repressor (AHRR) is a negative feedback regulator of AHR signaling by competing with AHR for its dimer partner, ARNT. The AHR also binds NF-κβ, and is a tumor suppressor gene. Four aryl hydrocarbon receptor (AHR) genes (AHR1, AHR1b, AHR2, AHR2b) and a single gene for the repressor (AHRR) have been identified in the Atlantic killifish, *Fundulus heteroclitus*. The role of AHR(s) in the ability of killifish to adapt to environments polluted with PCBs and PAHs has been the subject of intense research. To date, data strongly indicate that AHR2 has the most prominent role in the killifish's ability to adapt to PCBs and PAHs, and primarily through genetic mutations in the ligand binding domain. Tissue-specific protein localization of the AHRs and AHRR in killifish have not been examined using monoclonal antibodies (mAb), and thus the impedance for us to generate a panel of mAbs. Monoclonal antibody 5B6 was generated against recombinant AHR2, mAb 7B8 against recombinant AHR1, and mAb 9R71 against recombinant AHRR. To generate non-cross reactive mAbs against recombinant AHR1b and AHR2b, synthetic immunogenic peptides were designed, yielding mAb 4A3-2 and mAb A2B-2, respectively. Using immunohistochemistry, we show that AHR2 is ubiquitously expressed in all tissues, and highly expressed in the liver, intestines, and brain. AHR2 is also highly expressed in some chemically induced liver tumors in fish sampled from the wild. Using mAb 5B6, we show that AHR2 is also detected in *F. Grandis*, a sister species. AHR1 was detected in the early stages of larval growth and confined to areas of intense bone formation. AHRR was detected in adult livers, and seems to be concentrated near, or within the bile canaliculi. To date, we have not detected AHR1b and AHR2b protein in adult killifish, though it is possible these proteins are expressed differentially during development. **1R15ES016905, **P42ES007381.*

PS 2352 Impact of Phenothiazine on *Fundulus heteroclitus*

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Phenothiazine (PTZ) is a heterocyclic thiazine compound used for industrial and medical purposes. Through environmental surveillance studies PTZ was found being discharged into a local river in Connecticut. Phenothiazine has been shown to act as an endocrine disrupting chemical. This study sought to identify sex specific hormone receptor changes, a marker for endocrine disruption, in *Fundulus heteroclitus* in response to PTZ. *Fundulus heteroclitus,* also known as mummichog, are small fish native to the Atlantic coast of the United States and Canada. They reside in brackish waters and can survive harsh toxic environments. This model organism is native to the polluted waters found in Connecticut. In this study fish were exposed to concentrations of phenothiazine of 0.5ppm, 1.0ppm, and 2.0ppm for one week. Following exposure brain, liver, and gonad tissues were harvested; cDNA was synthesized; and mRNA expression was assessed for six different hormone receptors. When compared to vehicle control (ethanol) differences in mRNA expression levels of hormone receptors were observed in various tissues from male and female fish. Many of the tissues assessed showed changes in expression level, while only female liver and testis showed no change. These results implicate phenothiazine as an endocrine disrupting compound to mummichog at environmentally relevant concentrations.

PS 2353 Monitoring Colonial Waterbirds as Indicators for Reproductive and Immunological Impairments at Contaminated Great Lakes Sites during 2010-19

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This monitoring program assessed effects of contaminants, primarily PCBs and PCDDs, on immune function and reproduction in fish-eating birds in the Saginaw Bay and River Raisin Areas of Concern (AOCs) and Grand Traverse Bay in 2010-19 under the Great Lakes Restoration Initiative and AOC programs of the US Fish and Wildlife Service and US EPA. Saginaw Bay sites included two herring gull colonies (Confined Disposal Facility (CDF) and Little Charity Island), two Caspian tern colonies (CDF and Charity Reef/L. Charity Island) and one black-crowned night heron colony (CDF). Herring gulls were studied in the River Raisin AOC at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie and on Bellow Island in Grand Traverse Bay. Reference sites were in the lower St. Mary's River (gulls on Pipe Island Twins and terns on Two Tree Island), on Tahquamenon Island in Whitefish Bay (terns), and on Chantry Island, Lake Huron (herons). Gull embryos were assessed during late incubation using a viability detector sensitive to heartbeat and movement. Relative risk ratios for embryonic nonviability were significantly elevated 2-3 fold at contaminated sites compared to the reference site (2.1 for the Saginaw Bay AOC, 2.6 for the River Raisin AOC, and 2.8 for Grand Traverse Bay). Infertility was the primary cause of nonviability at the reference site. Elevated infertility and mortality contributed to nonviability in contaminated sites. Deformities associated with PCBs and PCDDs were found in several individuals at contaminated sites (3 gull chicks at Monroe, 2 tern chicks on L. Charity, 3 gull embryos on the CDF, 1 gull embryo on L. Charity, and 1 gull embryo on Bellow). Chick productivity in terns in Saginaw Bay (mean of 0.76 chicks/nest) was signifi-

cantly below that of reference sites (1.19 chicks/nest). In the River Raisin AOC, productivity of gull chicks was poor in 4 of 10 years, with complete reproductive failure in 2010. In gull chicks the mean phytohemagglutinin (PHA) skin response for T-cell mediated immunity was suppressed 55-56% at both AOCs and 50% in Grand Traverse Bay. This response was suppressed 49% in terns and 39% in herons in Saginaw Bay. Mean antibody responses in gull chicks at the River Raisin AOC and in Grand Traverse Bay 1.6-2 fold lower than at the reference site. Ongoing immunological and reproductive impairments at these contaminated sites are consistent with the effects of persistent pollutants such as PCBs and PCDDs.

PS 2354 Occurrence and Toxicity of Metals and Polycyclic Aromatic Hydrocarbons in Marine Sediments from Cartagena Bay and Grand Marsh of Santa Marta (Ramsar Site), Colombia

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Cartagena Bay (CB) is considered an important industrialized site in Colombia. The aim of this study was to analyze the presence of metals of toxicological importance and polycyclic aromatic hydrocarbons (PAHs) in marine sediments from CB and in the Grand Marsh of Santa Marta (GMSM), a Ramsar coastal lagoon, used as a reference site (RS). The samples were collected in 15 stations (12 in CB and 3 in the GMSM) during dry and rainy seasons. In addition, the *in vitro* cytotoxicity of methanolic extracts from gathered sediments was assessed using HepG2 cells. The characterization of the sediments from the sampling sites was performed through the analysis of metals (Cr, Cu, As, Cd, and Pb) by Inductively Coupled Plasma Mass Spectrometry (*ICP-MS*), Hg was measured using a Hg analyzer, and PAHs were analyzed by capillary Gas Chromatography-Mass Spectrometry in Selected Ion Monitoring mode (GC-MS/SIM). Most contaminated sites in Cartagena Bay corresponded to stations related to repair and maintenance of ships, with high concentrations of Cr, Cu, As and Cd. Stations receiving inputs from petrochemical and fertilizer plants displayed high content of Pb. Maximum Hg levels were obtained near an extinct chlor-alkali plant. At least 70% of the samples from CB presented Cr, Cu, and As levels that were greater or equal to the Threshold Effect Level (TEL) and the Effects Range Low, suggesting adverse biological effects could occur occasionally. Potential ecological risk values revealed that Hg and Cd may generate deleterious effects on aquatic life. The Bay was classified as a site with low to moderate PAHs pollution. These compounds were especially observed in a station near a petrochemical industry. PAH concentrations in some stations from CB were greater than the TEL, while in the GMSM none of the stations exceeded this guideline. Some sediments from CB were able to reduce cell viability (<50%) in HepG2 cells. The data highlight the need to investigate the ecotoxicological implications of the evaluated pollutants in the environmental quality of these ecosystems. *Grant FP448442-197/2017 (COLCIENCIAS-University of Cartagena), COLCIENCIAS 567-2012, the General System of Royalties of Colombia, SGR, Government of the Department of Bolivar and University of Huelva.*

PS 2355 Clinical Syndromes of Mushroom Poisoning-A Five-Year Experience in North East India

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Mushroom poisoning is one which is frequently encountered in the tribal areas of the country of India as wild mushroom forms part of the diet in the ethnic tribes of the country. However at the same time a huge variety of these wild mushroom are known to be poisonous and one of the cause of fulminant hepatitis for which the definitive treatment being liver transplant .The case reports of mushroom poisoning has been reported in North India and South India among the tribes of the areas with the intake of wild mushrooms unintentionally. In the north east India little is known about the type of syndrome presentation in patients with mushroom poisoning and little awareness is being provided about the non edible mushroom in this region. Here we are prerenting the clinical syndrome which we have encountered in the last 5 years from 2015 till August, 2019 in our tertiary hospital of the state of Meghalaya, which is a situated in the north eastern part of the country. All patients admitted to the hospital with history suggestive of mushroom poisoning were included as cases January 2015 to August 2019 and all relevant information were recorded in a structured performa. A descriptive analysis of all collected variables was carried out. Over 39 cases of mushroom poisoning presented to our hospital in the last 5 years, mainly in the the raining season (March- May) and autumn season (August -October). All cases were from the tribal areas of the four neighbouring 4 districts of the state. The wild mushrooms were self picked from the forest. 25 patients had developed early symptoms (less than 6 hours) and were less fatal, however 16 patients developed the symptoms after 6 hours of consumption, and were more seriously ill out of which 6 cases expired due to acute hepatic failure. The clinical syndrome presentation were acute gastroenteritiswith liver failure in 11 cases (suspected *Aminata* poisoning), 20 cases of acute gastritis and gastroenteritis (suspected mushroom species *Chlorophyllum molybdites*, *Clitocybe nebularis*, *Omphalotus illudens*), one case of Acute renal failure (suspected *Cortinarius violaceus* species), one case showing Disulfiram like reaction (suspected *Coprinus atramentarius*, *Clitocybe clavipes* species), 5 cases of Cholinergic mushroom poisoning (suspected *Citocybe dealbata*, *O. illudens*, *Inocybe fastigiata* species) and one case presented only with migraine. The mortality from mushroom poisoning resulted mainly due to fulminant hepatitis in 6 cases. Conclusion: With the five years observational study of mushroom poisoning in this part of the country the cause is mainly due to consumption of wild poisonous mushrooms, with amanita species type being responsible for most of the fatalities. From the clinical syndrome presentation and on visiting the affected areas, the *Aminata* species seems to be common, a variety of the other poisonous mushroom are also present as evident from the clinical presentation. The *Aminata* like species has been identified at the affected villages with our insight to their anatomical structure. With this study we aim to carry out toxicological analysis of the blood and urine samples of patients we present in future mainly for the amatoxin.

PS 2356 The ECOTOXicology Knowledgebase: Updating Literature Search and Review Processes for Identifying and Curating Toxicity Data for Risk Assessments

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The ECOTOXicology Knowledgebase (www.epa.gov/ecotox) is a nationally and internationally recognized source of curated single-chemical toxicity data for aquatic life, terrestrial plants, and wildlife. This publicly available database provides industry, federal and state regulators, and researchers with a cost-effective, non-duplicative means of locating high-quality ecological effects data to support chemical decision making. Through its 30+ year history, ECOTOX has developed systematic and transparent procedures to conduct comprehensive literature searches, title/abstract screenings, application of acceptance criteria, and data extraction of all pertinent study and effects information. The current rate at which chemicals are reviewed and data evaluated requires modernization and improved efficiency in the well-established ECOTOX review protocols, as well as to increase effectiveness of the user interface and interoperability across multiple sources of toxicity, chemical, and species data. Here we present efforts to refine and implement tools to improve current procedures, integrate ecological data with human health evidence mapping, and incorporate information of study quality into ECOTOX. Steps in the literature search process now include consistent application of search terms which allow resulting references to be used for evidence mapping, systematic reviews, scoping documents, risk assessments, and regulatory decisions. These efforts were followed by development and application of semi-automatic processes to document literature search terms, and modification of literature acquisition and systematic review tools. Further efforts to improve efficiencies have incorporated study quality information through identification of those used for regulations (e.g., Water Quality Criteria documents, EFED Risk Assessments). Finally, new and enhanced additions to the user interface will enhance the interoperability with other databases and tools. The updated functionality and interoperability will provide the regulated industry and researchers more effective methods to search and use existing toxic effects data to determine thresholds and conduct risk assessments.

PS 2357 Consumption of Contaminated Aquatic Species Alters the Mammalian Gut Microbiome

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Strong evidence suggests that fish and crabs inhabiting the Passaic River/ Newark Bay area, a superfund site in NJ, are contaminated with toxic agents including heavy metals and polycyclic and polychlorinated aromatic hydrocarbons. Despite published warnings to avoid consumption of contaminated fish, this practice continues in high numbers, particularly amongst anglers and their families in low-income communities. We hypothesized that consumption of "dirty" fish produces alterations in the GI tract microbiome. The GI tract microbiome encompasses metabolic, immune, and endocrine com-

munication and function in the host. It contributes to the maintenance of health, and when altered, can significantly influence susceptibility to, and phenotypes of diseases like CVD, obesity, and diabetes. While microbiota disruption is common in humans, the response to environmental stressors like dioxin, PCBs, and heavy metals has not been well characterized. To test our hypothesis, we utilized mice that were fed a 7-day baseline diet. This was followed by 10 days of autoclaved diets supplemented with a 2.5% or 7.5% "contaminated" or "clean" eel, sourced from the Passaic River or pristine Upper Delaware River, respectively. 16s ribosomal RNA was isolated from feces and sequenced at ~10,000 reads per sample using Illumina MiSeq. Principal Component Analysis (PCA) was used to examine the effects of diet and sex on GI tract microbial diversity in the mice. On day 1 of the dirty eel supplement, the community structure began to diversify. By day 9, a significant difference in microbiota makeup was seen in mice fed the 7.5% dirty eel diet. This effect was sex- dependent and the greatest changes were seen in male mice. These data demonstrate that consumption of contaminated aquatic species alters the mammalian gut microbiome. A similar experiment was conducted to explore microbiome changes in mice exposed to e-cigarette aerosols. Together, our data suggest that everyday environmental exposures can result in significant composition shifts of key bacterial communities that maintain host health.

2358 Toxicogenomic and Reproduction Effects in *Tribolium castaneum* Herbst after Exposure to Volatile Aromatic Compounds

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Volatile organic compounds (VOCs) such as naphthalene and benzene are widely used. Naphthalene is still being employed as a moth repellant; it is sold at supermarkets, and benzene is employed in diverse chemical and industrial processes. The aim of this study was to evaluate the toxicological effects of these VOCs against *Tribolium castaneum*. Exposure of adult insects at different concentrations of naphthalene (0, 40, 80, 120, 160 and 200 mg/L air) and benzene (0, 40, 80, 120, 160 and 240 µL/L air) were impregnated on filter paper disks, and placed on the underside of the screw cap of a glass vial. Ten insects were introduced in each vial without media before the cap was screwed tightly. The number of dead insects was counted after 24 and 48 h. LC_{50} values were calculated. Also, adult insect two-week-old were fed a diet of ground oatmeal and oat flakes 70:30, supplemented with naphthalene. Rearing of insects was performed in a glass jar with a maximum of 40 animals. The crops from insects were reviewed every 4 days, evaluating the number of larvae, size and observation of abnormalities. The LC_{50} for naphthalene and benzene to 48 h of exposure were 72.52 mg/L and 115.87 µL/L, respectively. At the highest concentration, these chemicals caused more than 80% of insect mortality. Furthermore, Real Time PCR analysis revealed the activation of oxidative stress and metabolism, and reproduction markers for insect exposure on naphthalene at concentrations of 80 mg/L at 4 hours of exposure. Adults exposed to benzene (80 µL/L at 4 hours) overexpressed genes related to neurotransmission, and reproduction. Naphthalene affected development producing abnormalities in larval and pupal stages. In the larval stage, it induced changes in the formation of limbs, swelling in the chest and lower sclerotized appendages. In the case of the pupae, induced occurrence of abnormality in the formation of limbs, wings, and abdomen. The data presented here provide evidence that VOCs inhalation in our experimental conditions is able to induce alteration of reproduction, oxidative stress, and death of the insect. *The National Program for Doctoral Formation (Colciencias, 567-2012).*

2359 Assessment of the Toxicological Effects of Young Landfill Leachate on *C. zizanioides* and Its Phytoremediation Ability

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Landfill leachates have a complex composition, which includes organic matter, ammoniacal nitrogen, heavy metals, among others. Without being treated, they could be an imminent source of contamination of surface and groundwater, thus, studies and advances in sustainable technologies are required for this purpose. This research assessed the potential of the macrophyte *Chrysopogon zizanioides* in the phytoremediation of young leachate. For this, morphological changes and metal uptake of Cadmium, Lead, Nickel and COD of the plant was measured after exposing it to concentrations of 25%, 50%, 75% and 100% of synthetic young leachate. Using the specified concentrations, plant growth was affected, decreasing in more than 50% for concentrations of 25% and 50%, compared to control. On the contrary, higher concentrations showed exponential growth during the experiments, plus, plants health and color improved in these concentrations. Metal uptake of Cadmium was up to 98,4%, 94,4%, 95,8%, 96,1%, Nickel 94,8%, 97,7%, 98,7%, 97,6%, and Lead 98%, 92.7%, 97%, 94%, suggesting the plant is a suitable model for phytorremediation of landfill leachate.

2360 Toxicological Effects and Phytoremediation of Zinc Oxide Nanoparticles in Hydroponically Grown Sorghum (*S. bicolor*) and Alfalfa (*M. sativa*)

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Nanomaterials have gained rising popularity in recent years thanks to their numerous applications and use thanks to their tiny size and high surface area, such is the case of Zinc oxide nanoparticles, which are among the most popular engineered nanoparticles as a result of its antioxidant and antifungal capacities. This rising popularity has led to their massive production and thus to an increase in its accumulation in the environment, especially in soils and consequently crops. In comparison to their bulk size, nanomaterials have different toxicological effects related to not only their size but also to their aggregation and sorption capacities, provoking their biopersistence and biomagnification. The goal of this study is to determine the toxicological effects which occur during the exposition to Zinc oxide nanoparticles (ZnONPs) using hydroponically grown Sorghum (*Sorghum bicolor*) and Alfalfa (*Medicago sativa*) as biomarkers, as well as its effects on the zinc uptake of both plants to evaluate their phytoremediation capacities. The seedlings were hydroponically grown using Hoagland's medium of half strength and exposed to concentrations of 0, 50 and 100 ppm of ZnONPs for a period of 7 days. Physiological data were taken for both plants before and after exposition, as well as data related to the water consumption of the plants. After the contamination stage, the remaining biomass was exposed to a digestion process for the quantification of the zinc content of the plant and the contaminated medium through atomic adsorption. All treatments presented symptoms of both chlorosis and necrosis, as well as root browning and growth inhibition. The alfalfa plant exhibited worse toxicological effects than sorghum, in both physical appearance as well as in growth, while sorghum, in spite of presenting growth inhibition and partial chlorosis, seemed to have a better resistance with the ZnONPs. The Zinc uptake in both plants was related to the ion release capacity and behaved similarly, however the alfalfa plant had higher uptake of the zinc ion due to its affinity with the metal, however this affinity led not only to an accumulation but also to an increase on its toxicity. Finally, the ZnONPs have moderate toxic effects in both plants, being more remarkable in the alfalfa plant but also giving hints of a remediation process making it an useful resource for zinc removal.

2361 Ultraviolet B Radiation-Induced DNA Damage in Southern Oak Tree Species

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Ultraviolet B radiation (UV-B) is a great cause for concern due to its role in DNA damage. This is especially true for plants because of their sessile nature. UV-B interacts strongly with DNA and this, among other things, results in the formation of two kinds of DNA damage products, namely, cyclobutane pyrimidine dimers (CPDs) and 6,4-photoproducts (6,4-PPs). If left unrepaired, these DNA lesions can impede replication and transcription, thereby causing cytotoxicity and *mutations*. In plants specifically, these DNA damage products can suppress photosynthesis and protein biosynthesis. This can lead to stunted growth, reduced yield, productivity and development. Towards understanding the nature of UV-B-induced DNA damage and the possible UV-B tolerance, we have collected leaf samples from six different broad leaf species on the Southern University campus in Baton Rouge, Louisiana during the spring (March and April), summer (June and July), and fall seasons (October and November) in the year 2017. In spring, new budding leaves, for summer, mature leaves and for fall senescing leaves were selected. In each season, the tree branches were clipped randomly from the most sun-exposed part. Following cleaning with wet wipes or 1% bleach (if required), the leaves were randomly selected, and their chlorophyll content was measured (Spad chlorophyll meter) to make sure all the leaves selected for analysis were in the same growth phase. The leaves (10-50) per species were placed in zip-lock bags and stored at -80ºC until further experimentation which included DNA extraction (Qiagen) and analysis of CPDs and 6,4-PPs (ELISA). The CPDS and 6,4-PPs were analyzed typically using 500 pg DNA. The results indicated that all six different oak species studied, namely, *Quercus virginiana* (southern live oak), *Q. acutissima* (sawtooth oak), *Q. nigra* (water oak), *Q. phellos* (willow oak), *Q. shumardi* (Shumard oak) and *Q. nuttalii* (nuttal oak) had evidence of DNA damage which varied with the tree species studied and the seasons (spring, summer and

fall) in which the samples were collected. This study gives an idea into better understanding of the UV-B tolerance developed by different species to cope with the intense and unrelenting solar UV-B radiation, especially in light of the enhanced UV-B radiation level due to ozone holes developed in the Earth's stratosphere.

2362 Skin Microbiome-Survey of Cosmetic Products on the US Market

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Sponsor: Z. Wang

With probiotics becoming widely commercialized in foods and drugs, we have started to see its appearance in cosmetics. From 2013-2017, the US Food and Drug Administration (US FDA) has observed an increasing number of cosmetics claiming to contain ingredients that may potentially impact the skin microbiome. Mintel Market Research, in this same time period, reports that the use of *LactoBacillus*-based probiotic skincare products increased by 98% in the US To gain a better understanding of this growing market, US FDA conducted an evaluation of US launched cosmetic products that target the skin microbiome between 2003 and 2018, using the publicly available Global New Product Database (GNPD) by Mintel. We found 255 cosmetic products marketed during this time frame claiming to use probiotics. In examining the data further, we found that probiotic skincare products in the US more than doubled in 2018 compared to 2017 (70 vs 29 products, respectively). Many of the products claim to use probiotics and probiotic-derived ingredients. Our analysis also shows that there are three distinct approaches being used by the cosmetic industry to target the skin microbiome: 1) probiotics (the infusion of live cultures), 2) prebiotics (non-viable nutrients intended to promote "good" skin microbiota), and 3) postbiotics (non-viable soluble factors or metabolic by-products secreted or released after bacterial lysis, such as ferments, lysates, filtrates or extracts). Interestingly, regardless of which approach is stated in the ingredient list (probiotic, prebiotic or postbiotic), approximately 90% of the 255 cosmetic products were found to contain postbiotics. Product label reviews showed that *LactoBacillus* is the genus most often used as the probiotic ingredient or probiotic derivative. While 93/255 (36%) "probiotic" products claim that they have no additives or preservatives, further analysis revealed that the majority (95%) contain a combination of different preservation systems.

2363 Optimized Long-Term Skin Organ Cultures to Assess Dermal Toxicity Risks

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Skin organ culture technology can play an integral role in all aspects of skin biology and skin damage research. Different skin models, such as 3D-reconstructed skin or standard 2D monolayer cell culture, have been used for biological skin studies and for ingredient or finished product testing. However, the major limitation of those models is the lack of skin native complex architecture because of containing at best 2 or 3 cell types. Skin native explants remain the relevant physiological model that best mimic *in vivo* situation, but skin explants can be cultivated only in a short-term period at 32°C. Therefore many efforts were made on developing an optimized culture medium for maintaining long-term skin explant/organ cultures and so that for providing a suitable *in vitro* model for prediction and classification of skin irritation, corrosion, sensitization as well as skin metabolism. Regarding irritation and xenobiotic metabolism studies, the well maintained and physiological skin organ culture can be a key condition. We aimed at developing a novel defined and animal component free skin culture medium 1) for long-term of skin explant culture, up to 2 to 5 days of transport/storage at 4°C followed by 14 days at 32°C with good skin structure integrity and physiological functions; 2) for an optimized *in vitro* skin culture model to predict dermal toxicity risks and metabolism capability. Results: We observed 1) a prolonged viability of skin explants and well-preserved epidermis-dermis junction in optimized medium up to 14 days; 2) a variations of impedance during transport/storage and culture in both media; 3) a good predictivity of induced irritation and a stable metabolic activity to the long-term skin culture compare to short-term skin culture. Optimized animal component free skin long term culture medium maintained a better preservation of explants up to 14 days of skin culture in comparison to that of the classical medium (3-5 days only). This optimized medium appears to be a relevant solution for long term-tested pharmaceutical and cosmetic products. The dermal toxicity and metabolism testing results obtained from the skin cultured in optimized long term medium are comparable to those of short term skin culture.

2364 An *In Vitro* Cell Model for Toxicity Studies and Pigmentation Regulation Utilizing hTERT Immortalized Neonatal Dermal Melanocytes

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Skin pigmentation is a complex process; melanocytes produce melanin and package it into melanosomes that are in turn exocytosed, then endocytosed by neighboring keratinocytes. Numerous genes play roles in controlling pigmentation at various levels of melanin production. Mutations in these genes are characteristic of multiple skin disorders, including hyperpigmentation, hypopigmentation, and mixed hyper-/hypopigmentation. Additionally, extrinsic factors secreted by the surrounding resident cell types also regulate the melanin expression in melanocytes. Human primary cells can be a useful model for elucidating melanocyte biology and their toxicity to dermal agents. However, primary cells have their limitations such as donor variability and limited lifespan. Consequently, a need exists for a more robust human cell model system for the study of skin pigmentation and agents which are toxic to melanocytes. In this study, we immortalized primary epidermal neonatal melanocytes by expressing human telomerase reverse transcriptase (hTERT) in cells that were isolated from a neonatal donor. The immortalized primary melanocytes were cultured continuously for more than 35 population doublings without any signs of replicative senescence, yet retained melanin production. The immortalized primary melanocytes maintained a consistent expression of the melanocyte-specific marker TRP-1, and lacked expression of the fibroblast-specific marker TE7. In addition, we demonstrate the capability of these immortalized primary neonatal melanocytes to transfer melanosomes to keratinocytes utilizing a 3D human dermal organotypic culture, and the ability to modulate melanogenesis with depigmentation agents and these agents' effects on cell viability. Taken together, the hTERT immortalized primary neonatal melanocytes described here provide a versatile *in vitro* cell model for the study of cosmetic agents, their toxicity and melanogenesis regulation.

2365 Tetramethylammonium Hydroxide (TMAH) Contamination on Human Skin Explants *Ex Vivo*

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Tetramethylammonium hydroxide (TMAH) is a quaternary ammonium compound and strong corrosive base. It dissociates into TMA^+ and OH^- ions, causing chemical skin/eye injuries and sometimes fatal systemic toxicity. Dermal injury from the OH^- ion may increase TMA^+ tissue penetration. This study was performed to evaluate general morphology effects of TMAH contamination on human skin explants *ex vivo*. With informed consent, 60 human skin explants were prepared from an abdominoplasty sample and preserved *ex vivo* in BIO-EC's Explant Medium at 37° C in a humid atmosphere containing 5% CO_2. Explants were divided into 13 groups: (1) untreated controls, (2-13), explants exposed to 25% TMAH solution for different time periods - 1, 5, 10, and 20 minutes, 1, 2, 4, 8, and 24 hours. TMAH was applied on a 9mm filter paper saturated with 30 µL. Duplicate explant samples were taken from all groups on Day 0, and compared with triplicate samples from all groups at 24 hours post-exposure. Samples were either fixed in buffered formol or preserved frozen at -80°C. Samples were prepared for histological evaluation. Sample preparation: dehydration and impregnation into paraffin using a Leica PEARL automatic dehydrator, then cut into blocks with a Leica EG 1160 coating station. 5 µm slices were prepared with a Leica RM Minot-type microtome and mounted on glass Superfrost® slides. Slides were stained with the Goldner variant of Masson's Trichrome. Optical microscopy was performed with a Leica-type DML-B or Olympus BX42. Photomicrographs were taken with an Olympus DLP72 and Cell software. After a 25% TMAH contact time of 1 hour, the general morphology of epidermis and dermis were totally altered. With longer contact times, 2 of 3 explants were very significantly altered. However, alterations were irregular (2 of 3 explants). Alterations were observed at 24 hours after a 20 minute contact time. Based on these results, for future studies in this model, a 25% TMAH solution contact time appears to be the best option and >3 samples could minimize possible sample heterogenicity.

2366 Increasing Dose Recovery from Unoccluded Static Diffusion Cell Studies

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Determination of percutaneous penetration is typically achieved using *in vitro* skin diffusion cell methodology. A number of protocols and guidelines have been published to ensure the reliability of results obtained by this method. Perhaps the most useful of these is the Organisation for Economic Co-operation and Development (OECD) guideline 428 and its associated guidance documents, which set the standard as to what is acceptable when conducting *in vitro* skin penetration studies using diffusion cells. These guidelines attempt to ensure that *in vitro* studies are carried out appropriately and aim to reduce variability from laboratories within different organisations. One of the guidelines concerns dose recovery and stipulates a recovery requirement of 100 +/- 10% of the applied dose. For unoccluded studies, using volatile chemicals, this is not feasible unless vapour capture methodology is used. The current work evaluated a charcoal filter system as a means of vapour capture. *In vitro* percutaneous absorption experiments were performed with Franz-type glass diffusion cells with an area available for diffusion of 2.54 cm^2. The receptor fluid used was 50% aqueous ethanol. A total of 8 cells were assembled for each test condition (control unoccluded; Tin foil occluded; charcoal filter). A 10µl droplet of 14C-methyl salicylate was applied to each piece of dermatomed human skin (500 µm thickness, obtained with informed consent from reduction surgery). Samples of receptor chamber fluid were removed at regular intervals up to 24 h post exposure. A full dose distribution was performed at study completion. The presence of the charcoal filter increased total recoveries from 30% for the unoccluded control to 95% allowing the study to meet OECD guidelines. The presence of the charcoal filter also increased the skin penetration of methyl salicylate relative to the unoccluded control. This increased penetration was not as pronounced as for the cells occluded with tin foil. It is important to understand the potential consequences of incorporating vapour capture technologies into diffusion cell systems. © *Crown copyright (2019), Dstl. This material is licensed under the terms of the Open Government Licence except where otherwise stated. To view this licence, visit http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3 or write to the Information Policy Team, The National Archives, Kew, London TW9 4DU, or email: psi@nationalarchives.gsi.gov.uk.*

2367 *In Vitro* Dermal Absorption of Carfentanil

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There is growing concern regarding potential occupational exposures to the ultra-potent synthetic opioid carfentanil. However, little data are available on the toxicity of carfentanil in humans, particularly for dermal exposures. To begin to address this, permeation of carfentanil formulated in three vehicles, water, ethanol, and hand sanitizer was measured under infinite-dose conditions in an *in vitro* static diffusion cell system using the EpiDerm™ (EPI-606-X) RhE model. The permeation rate was fastest for carfentanil in water (3.9×10^{-3} cm/hr), followed by hand sanitizer (1.2×10^{-3} cm/hr), and slowest for carfentanil in ethanol (0.2×10^{-3} cm/hr). In both ethanol and hand sanitizer, a lag-time between exposure and permeation of approximately 1.5 hours was observed, while lag-time in water was approximately half an hour. Flux at steady-state was greater at 50.6 µg/ml than at 5.3 µg/ml for both water and ethanol; however, the percent of dose absorbed did not differ between doses for either vehicle. Slight differences in percutaneous permeation of carfentanil were observed between two brands of hand sanitizer, likely due to differences in relative proportion of alcohol and skin penetration enhancers. These data indicate that small skin exposures may not result in rapid, significant toxicity as previously reported.

2368 Suitability of *In Vitro* Skin Assays for Acrylic and Methacrylic Monomer-Based Adhesives

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In vitro skin irritation assays based on reconstructed human epidermis (RhE) models are widely accepted to accurately identify non-corrosive and corrosive chemicals and mixtures in accordance with UN GHS. It is known that undiluted acrylic and methacrylic monomers are often either corrosive or irritating to skin due to their acidity. To assess the skin corrosion potential of certain monomers and adhesives, skin corrosivity tests according to OECD Test Guideline (TG) 431 were performed using the Open Source Reconstructed Epidermis (OS-REp) model. Technical proficiency was demonstrated for all twelve Proficiency Substances listed in OECD TG 431. All substances, amongst them six acids, were correctly classified and subcategorized as Cat. 1A or 1B/C corrosives. None of the monomers interfered with the OECD TG 431 assay by unspecific MTT reduction or pH-dependent shift of the MTT formazan absorption maximum. Existing *in vivo* data demonstrate that acrylic acid, methacrylic acid, and 2-hydroxyethyl methacrylate phosphate are skin corrosives. However, already a concentration of only 2% acrylic acid and methacrylic acid, respectively, triggered a corrosive classification when tested in OECD TG 431. In addition, the irritant compound methyl methacrylate was incorrectly predicted as corrosive. All tested adhesives with methacrylic acid and methyl methacrylate yielded corrosive results. No correlation was found between the content of corrosive ingredients and the extent of corrosive effect. Surprisingly, histopathological evaluations and determination of cell viability by means of lactate dehydrogenase measurement do not always confirm the corrosive results of OECD TG 431. The current study demonstrates that the OS-REp skin model can be used for corrosivity testing in OECD TG 431 to assess product safety. Our results also indicated that adhesives materials such as acrylic and methacrylic monomers might fall outside the applicability domain of this test guideline.

2369 Formal Validation of the GARD™potency Assay for Subclassification of Chemical Skin Sensitizers—Ring Trial Results of Predictive Performance and Reproducibility

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The advancement of non-animal approaches for hazard assessment of skin sensitizers have generated a variety of alternative assays that covers relevant aspects of the known sensitization mechanisms, and that exhibits discriminatory properties competitive with those of accepted *in vivo* methods. However, hazard identification is rarely sufficient and information permitting the relative ranking of chemicals' skin sensitization potency is desired. For example, the globally harmonized system of classification and labelling of chemicals (GHS/CLP) extends the binary hazard assessment with a qualitative subcategorization to distinguish weak skin sensitizers from strong. Though substantial efforts have been made towards developing alternative methods for potency assessment, none have gained regulatory acceptance. In contrast, the regulatory accepted *in vivo* method, the murine Local Lymph Node Assay (LLNA), provides a quantitative readout that is relevant for potency assessment. Considering the ethical aspects and the implementation of new regulations, the development of improved alternative assays is of high priority. The genomic allergen rapid detection (GARD™) is an *in vitro* testing platform for assessment of chemical sensitizers. Though originally developed for skin sensitization hazard assessment, recent developments allowing for subcategorization of chemicals in accordance with GHS/CLP categories showed promising results. To validate the GARD™ assay for assessment of skin sensitizer potency (GARD™potency), a ring-trial study was carried out in compliance with the OECD guidance document No. 34. Reproducibility estimates of the assay ranged between 62.5% and 88.9% within participating laboratories. Between laboratory reproducibility was calculated to 61.1%. The predictive performance estimates ranged between 76.5% and 94.4% for individual laboratories, and the cumulative accuracy was summarized to 88.0%. The results suggest that the GARD™potency assay could be a valuable tool for hazard characterization of skin sensitizer potency. The assay is included in the OECD Test Guideline Programme (TGP 4.106) and is suitable for submissions under REACH.

2370 Elucidating Mechanism of Toxicity of Contact-Sensitizing Hair Dye Components by *In Silico* Approach

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More than 15% of the general population have allergic contact dermatitis as a clinical manifestation of contact sensitization, making this skin disease a major health problem. Screening for contact-sensitizing chemicals is highly relevant, particularly in dermatology. Ethical considerations, need for high-throughput screening and legislation (e.g., for cosmetics in the European Union) are limiting the use of animal tests for this purpose. Although hair dye substances, that are strong or extreme sensitizers, are very common in consumer hair dye products on the European market, at present it is not possible to accurately estimate the overall burden of hair dye allergy in dermatitis patients. Dermatitis patients are generally patch tested with one substance only, namely p-phenylenediamine. The goal of the study was to develop knowledgebase and software tool that can be used to predict potentially harmful interactions between chemicals as an alternative to animal test

methods. From the public domain and proprietary company datasets, we collected data on chemicals contained in a variety of consumer products such as health and beauty aids, household products, drugs and food. We generated computable networks of interacting chemicals, genes, proteins, pathways, and biological effects, providing extensive information on chemicals and their biological effects on organisms. To validate our approach, we used the three ingredients commonly used in permanent hair dye formulations (p-phenylenediamine, PPD; Resorcinol, RES and hydrogen peroxide, H_2O_2), and demonstrated how simultaneous exposure may result in one chemical augmenting the toxic effects of the other. Our computational tool shows that RES and PPD target molecules that are involved in inflammation and allergy reactions like cytochrome P450 protein CYP1A1, CYP3A4, prostaglandin synthase PTGS2, chemokine receptors CXCR4, CCR6 and cytokines IL8, IL5. Further, our system identified correlation between these two chemicals and various skin conditions such as skin sensitization and skin inflammation. Bioinformatics analysis provides the underlying mechanism of how exposure to these hair dye components can induce skin sensitization, and assumed explanation of additive and/or synergistic effect observed with these three chemicals.

2371 Effectiveness of h-CLAT, an *In Vitro* Skin Sensitization Test Method, in Evaluating Respiratory Sensitizers

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Respiratory sensitization induced by chemicals is an important occupational and public health issue because it is associated with allergic asthma or other pulmonary symptoms. However, there are no validated test methods which can identify respiratory sensitizers. In this study, in order to investigate behavior of respiratory sensitizers in skin sensitization test method, 14 known respiratory sensitizers such as diisocyanate, acid anhydride, platinum salts, aldehyde, ethylenediamine, reactive dye and flavoring agent were tested with h-CLAT. h-CLAT (listed in OECD TG442E) is an *in vitro* skin sensitization test, which evaluate dendritic cell activation by measuring the expression of the cell surface CD86 and CD54 antigens in THP-1 cell line. As a result, 7 of 14 respiratory sensitizers were resulted in positive with h-CLAT. Especially in diisocyanates which are very important industrially compounds as polyurethane materials, all four respiratory sensitizers were positive. Furthermore, for 3 acid anhydrides which showed negative results in h-CLAT, we also performed modified h-CLAT (short time exposure method using liquid paraffin) and phthalic anhydride was positive with the modified method. Among 7 false negatives in h-CLAT, at least 4 chemicals are known to be positive in DPRA (other *in vitro* skin sensitization test method listed in OECD TG 442C). These results suggest that h-CLAT could be useful non-animal test method with respect to respiratory sensitizers if combined with other test methods like DPRA.

2372 Applicability of GARD™skin for Accurate Assessment of Challenging Substances in the Context of Skin Sensitization Testing

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Proactive identification of chemicals with skin sensitizing properties is a key toxicological endpoint within hazard testing. To date, several *in vitro* assays for assessment of skin sensitizers have been proposed, some of which have gained various levels of regulatory acceptance. However, certain subsets of the chemical space relevant for testing remain challenging to accurately assess. Such challenging substances may include substances with severe membrane-damaging properties, pre- and pro-haptens, or substances with high logP-ratios. The GARD™skin assay (Genomic Allergen Rapid Detection) is a state-of-the-art non-animal-based testing strategy which evaluates the transcriptional patterns of endpoint-specific genomic biomarker signatures in a human dendritic-like cell line following exposure, in order to provide machine learning-assisted classifications of tested substances. The assay was recently subjected to a formal validation procedure (OECD TGP 4.106) and reported reproducibility between laboratories of 92%, as well as a predictive accuracy of 94%. Here, we provide data demonstrating the applicability of GARD™skin in such a challenging chemical space, using an overlapping subset of chemicals previously tested in an integrated tested strategy (ITS) based on validated, aqueous *in vitro* assays, as well as in a series of RHE-based assays. In a case study covering nine unique challenging substances, GARD™skin exhibits a predictive accuracy of 78%, compared to human data. Corresponding performances of RHE-based models ranged between 27-73%, while the ITS had an accuracy of 45%. In conclusion, we demonstrate that GARD™skin constitutes an accurate method for assessment of skin sensitization, also in the context of substances otherwise regarded as challenging.

2373 Development and Application of a Next-Generation Risk Assessment Framework (NGRA) for the Evaluation of Skin Sensitization of Cosmetic Ingredients

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Cosmetics Europe has compiled a database of non-animal skin sensitization data and has used it to evaluate the defined approaches included in the OECD IATA case studies project. In the next step we have run case studies where the participants were asked to use these data as basis for their safety assessment. The sharing of case studies at a workshop demonstrated that the available Defined Approaches (DA) can form an integral part of the New Generation Risk Assessment (NGRA) but alone are insufficient to draw a conclusion. Due to the variety of risk assessment needs and complexity in types of information that should be integrated in the skin sensitization risk assessment, a framework is needed to support NGRA for cosmetic ingredients. Here we present an NGRA framework for the evaluation of skin sensitization which is based on the classical risk assessment workflow and relies upon the principles developed by the International Cooperation on Cosmetics Regulation (ICCR). The key elements are that the risk assessment should address a clear question. It must be human relevant, exposure led, hypothesis driven and designed to prevent harm. A tiered, iterative approach should be applied, starting with a thorough review of all of the available existing information. Available data from non-animal test methods can be utilized within any of the available DA, the choice of DA applied might depend on the information available and the risk assessment question. To demonstrate the practical application of the framework we performed a series of case studies with a diverse set of chemicals including lactic acid and methyl dibromo glutaronitrile that were based only on *in vitro* and *in silico* data. This exercise demonstrated that the framework is useful to structure the next generation risk assessment and it increased the confidence in decision making using *in vitro* and *in silico* data. The remaining challenges, as identified via the case studies, will be discussed. In conclusion, significant progress has been made in development and application of non-animal approaches in NGRA for skin sensitization. We have shown that the workflow presented here can help harmonize the risk assessment process while allowing sufficient flexibility for integrating different data and a diverse chemical space.

2374 A Risk Assessment of the Skin Sensitization Induction Potential of Personal Care Product Preservatives Ethylhexylglycerin and Phenoxyethanol

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Preservatives are commonly added to personal care products to prevent microbial contamination as well as to prevent potential health effects associated with use of adulterated products. However, it is important to understand if the addition of these preservatives is associated with health risks. The goal of this analysis was to perform a quantitative risk assessment to evaluate the skin sensitization potential of two preservatives used in a commercially available hair cleansing conditioner product. Specifically, ethylhexylglycerin and phenoxyethanol are preservatives used in a hair care product that was alleged to cause adverse events in users including skin rashes, redness, and irritation. As part of our assessment, we calculated an estimated daily consumer exposure using the amount of hair cleansing conditioner applied per application, the number of applications per day, a maximal dermal exposure parameter, a rinse-off retention factor, the maximum concentration of the preservatives in the product, and the skin surface area of the scalp. We calculated margin of safety (MOS) values by comparing the estimated consumer exposure levels with the no expected sensitization induction level (NESIL) for ethylhexylglycerin and phenoxyethanol (derived from the dermal sensitization threshold

[DST] approach). A sensitization adjustment factor of 100 was applied to the NESIL prior to the MOS calculation (10 for human variability, three for matrix variability, and three for use variability). The MOS for both preservatives was above 1.0 (range of 1.1 to 9.0), indicative of a low likelihood of skin sensitization induction. This evaluation provides evidence that use of the products under the examined exposure scenarios would not increase a consumer's risk of skin sensitization induction due to exposure to the preservatives ethylhexylglycerin and phenoxyethanol.

2375 Evaluation of Irritation Potential of Topically Used Compounds in High-Throughput Reconstructed Human Epidermis and Bio-Printed Full Thickness Skin Tissue

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The use of *in vitro* model as an alternative to animal testing for the safety evaluation of both products and ingredients in cosmetics and consumer products is already mandated by law in the European Union and other countries. The study presented here aims to assess the irritation potential of topically-used compounds in skin cellular models with different complexity, ranging from 2D monolayer culture of keratinocytes to 3D bio-fabricated skin tissue equivalents. A set of 451 topically-used compounds was tested for cytotoxic effects in primary neonatal keratinocytes and immortalized human keratinocytes. The 46 most cytotoxic compounds in the monolayer culture systems were further tested in biofabricated reconstructed human epidermis (RhE) and full-thickness skin (FTS) tissues in a 96-well plate format. Tissue viability, transepidermal electrical resistance (TEER), and secretion of cytokine IL1α and IL18 as markers of inflammatory and cellular immunity responses were measured after compound treatment. Among the known irritants, high concentrations up to 200 μM of methyl violet and methylrosaniline chloride decreased tissue cell viability, TEER and increased IL1α as expected, but low concentrations of these compounds increased IL18 without affecting IL1α secretion or reducing tissue viability or TEER in FTS, which suggests concentration-dependent sensitization and irritation potentials for these two compounds. Results from this study provide initial datasets to evaluate the effects of topically-used compounds in *in vitro* and *ex vivo* models and benchmark different endpoints to identify dermal hazards.

2377 Human Epidermal Cell Responses to Wood Combustion Products: Cornified Envelope Formation

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Air pollution poses many serious risks to public health. As a barrier between the human body and the external environment, the skin is constantly challenged by many air pollutants, including polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), particulate matter, and oxidants. Those air pollutants can have considerable effects on skin health and have been associated with skin aging, inflammation and cancer. Despite numerous studies on such effects, our knowledge regarding the mechanisms of action remains limited. We used cultured human epidermal keratinocytes treated with commercial liquid smoke (LS) to further investigate the toxicity of air pollution, specifically wood smoke, on epidermis. Produced by condensation of wood smoke from smoldering wood chips or sawdust, LS has been used widely as a seasoning to add a smoky flavor to recipes. Despite the refining process, LS contains many potentially harmful compounds found in wood smoke, such as VOCs. In our study, cornified envelope (CE) formation was assayed as a marker of keratinocyte cell death. Normally, formation of CE is the final step of keratinocyte cornification, a well programmed cell death in the epidermis. CEs, consisting of cross-linked proteins, are key structures for skin barrier function. Improper CE formation is a major cause of debilitating ichthyosis and may contribute to atopic dermatitis. To this end, keratinocytes were first treated with Mesquite or Hickory LS diluted 100- to 10000-fold in culture medium. At a dilution of 1 to 300, ≈20% of the cells (which were killed) formed CEs in one day while, at a dilution of 1 to 100, nearly all the cells formed CEs. However, the CE formation did not appear to occur through a normal cornification process, considering that expression of genes ordinarily involved in cornification were hardly altered by LS exposure. Moreover, Caspase 14, a protein that has been associated with cornification, was not activated. Finally, our initial proteomic experiments point to alteration of the CE protein profile by LS exposure, which could disrupt barrier function. Such effects provide a mechanism by which air pollutants could aggravate skin diseases.

2378 The Effects of Cleaning Petroleum Solvent or Tetrachloroethylene on the Expressions of Cytokines of Human Keratinocyte Cell Line Activated by Trychophytin

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Cleaning petroleum solvent has been widely used for dry cleaning. Once, tetrachloroethylene was also used for dry cleaning in Japan. In several reports, remained these cleaning solvents was considered as causes of skin damages including dermatitis. In addition, environmental factors such as fungi has been focused for skin damage. Trychophytin, fungal antigen produced by Trichophyton, activates keratinocytes resulting in the increase in the productions of cytokines such as interleukin-8 (IL-8) and tumor necrosis factor α (TNFα). It is of interest whether or not activated keratinocytes are affected by cleaning solvents resulting in the alterations in mRNA expressions in cytokines. In this study, a human keratinocyte cell line HaCaT was used as an *in vitro* model for human skin. The effects of cleaning petroleum solvent or tetrachloroethylene on the mRNA expressions of IL-8 and TNFα in activated HaCaT cells by Trychophytin were evaluated to elucidate the toxic effects of cleaning petroleum petroleum solvent or tetrachloroethylene on skin. The HaCaT cells were activated by Trychophytin at 10^{-5} PNU/ml (1PNU = 0.01 mg of phosphotungstic acid precipitate) and exposed to cleaning petroleum solvent at 0, 25, 50, 100 μg/ml or tetrachloroethylene at 0, 50, 125, 250 μg/ml for 6 hours in 24-well cell culture plates. The mRNA expressions of IL-8 and TNFα were evaluated by real-time PCR. The mean values of relative expressions of TNFα among the activated cells were exposed to cleaning petroleum solvent were significantly lower than that among the control. The mean values of relative expressions of TNFα among the activated cells were exposed to tetrachloroethylene at 50 μg/ml was significantly higher than that the control and the cells exposed to 250 μg/ml. There were no significant differences in the IL-8 expressions among the activated cells exposed to cleaning petroleum solvent or tetrachloroethylene. It is suggested that the effects of cleaning petroleum solvent or tetrachloroethylene on the mRNA expressions of on the cytokine expressions of activated keratinocytes were not strong. The reported skin damages by these chemicals may be due to cytotoxic effects of these chemicals.

2379 The Effects of UVR Filters in Commercial Sunscreens on AhR Signaling in Mouse Skin

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Ultraviolet radiation (UVR) is a persistent part of the environment for most humans. UVR has the benefit of catalyzing the production of vitamin D in the skin and is used clinically to ameliorate a variety of inflammatory skin conditions (i.e. psoriasis, jaundice). However, UVR is also well known for its negative health effects, particularly its production of sunburns and DNA damage that may promote skin cancer. UVR filters formulated into commercial sunscreens are used to reduce these negative consequences, however, there is a growing body of literature suggesting that they can have off-target effects. The majority of this literature has examined hormone-disrupting effects of UVR filters. Based on their chemical composition, we were curious as to whether or not UVR filters could modulate Aryl hydrocarbon receptor (AhR) signaling. The AhR is a ligand activated transcription factor important for metabolism of exogenous and endogenous compounds, immune function, and cell proliferation. Our *in vitro* studies found that the UVR filter octinoxate (OT) potentiates the mRNA expression of AhR target genes greater than what AhR ligands alone elicit, and that this is due to inhibition of the cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) genes. Our current efforts focus on determining if this phenomenon occurs *in vivo* and elucidating the potential consequences of it. We discovered that, in mouse skin, co-treatments of OT and the known AhR ligand 6-formylindolo-3,2b-carbazole (FICZ) lead to elevated levels of CYP1A1 and CYP1B1 mRNA levels that are greater than what FICZ alone elicits. FICZ is thought to be produced in the skin after UVR exposure, and UVR has been shown to activate the AhR and lead to increases in various target gene expression. Franz diffusion cell experiments were conducted to measure the permeability coefficient of OT through mouse skin and we discovered that OT permeates at approximately the same rate as nicotine. We additionally examined if co-treatments of UVR and OT had an impact on skin barrier. We measured transepidermal water loss (TEWL) on mice that were exposed to UVR with or without OT and found that co-treatments did not significantly impact TEWL levels 4 days after exposure. While there is still more to be done, our preliminary results suggest that the potentiation effect we observed *in vitro* also occurs *in vivo*, which may have important implications for human use. We are currently examining the effects of OT on contact hypersensitivity responses and cytokine production in mice.

2380 Polycyclic Aromatic Hydrocarbon Benzo[a] pyrene Exposure Exacerbates Skin Inflammatory Pathophysiology in a Mouse Psoriatic Model

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A rapid increase in the global prevalence of skin inflammatory diseases like psoriasis cannot be attributed to genetics alone. Environmental factors including major ubiquitous organic pollutants like polycyclic aromatic hydrocarbons (PAHs) could contribute to their pathophysiology. Benzo[a]pyrene (BaP) is one of the PAH that is generated mainly from cigarette smoke, wood-burning, automobile exhaust etc. The molecular mechanisms that lead to inflammatory skin diseases following exposure to BaP are not well elucidated, though there are suggestions that these responses could be mediated by aryl hydrocarbon receptor (AhR), a xenobiotic sensor. To investigate the effect of BaP exposure on skin inflammation in a mouse psoriatic model, the dorsal skin of naïve C57BL/6 mice was shaved (48h prior to exposure) and exposed to 62.5mg of 5% imiquimod (IMQ) cream once daily for five days. For assessing the initiation or exacerbation of psoriasis from exposure to BaP, mice were exposed 64µg BaP in 50µl acetone for five days before IMQ application [BaP+IMQ] or for five days together with the IMQ [(BaP+IMQ)] application. Following the analysis and scoring of clinical lesions, mice were sacrificed, and skin sections were analyzed for inflammation-related histopathological and molecular changes. BaP exposure together with IMQ exacerbated IMQ-induced psoriatic inflammatory symptoms including the skin bi-fold thickness, epidermal and dermal thickness, hyperkeratosis, dermal fibrosis, neutrophil infiltration, neutrophil degeneration and mast cell degranulation. Based on these results, we are currently analyzing and quantifying the effect BaP on IMQ-induced alterations in neutrophil infiltration (myeloperoxidase assay), macrophages (F4/80 staining), inflammatory cytokines and metabolites (metabolomics). In other studies, defining the role of AhR in BaP-induced biological, molecular, and metabolic alterations in mouse skin, concomitant treatment with AhR antagonist CH-223191 was given before BaP+IMQ treatment. The antagonist treatment before the (BaP+IMQ) treatment abrogated the BaP-caused increase in epidermal and dermal thickness in psoriatic mice. The effect of the AhR inhibitor on other biological and molecular psoriatic parameters that are exacerbated on exposure to BaP are also being analyzed to decipher the role of AhR in BaP-induced and/or exacerbation in inflammatory skin diseases like psoriasis.

2382 Call for a Multi-Product Testing Strategy to Evaluate Potential Adverse Dermal Effects

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The average female and male uses 12 and 6 personal care products each day, respectively, with potential daily exposure to an average of over 100 unique ingredients. The considerable use of personal care and cosmetic products highlights the importance of product safety. Currently, the US FDA does not prescribe product safety testing requirements for either personal care or cosmetic products or their individual ingredients before the products enter the market. Moreover, cumulative or aggregate exposures that lead to additive or synergistic effects are rarely discussed or evaluated. The goal of this study was to evaluate a multi-product testing strategy and propose next steps to help establish such a strategy. Two products (one cleansing conditioner and one hair dye) with 14 and 19 unique ingredients, respectively, were selected for this study. Dermal irritation was selected as the endpoint of interest based on the intended use of the product. *In silico* evaluation using the OECD QSAR Toolbox was performed to identify ingredients with potential dermal irritation hazard. Subsequently, *in vitro* evaluation using the OECD 439 guideline test was performed with each test article alone and in combination (50/50 ratio) to predict *in vivo* dermal irritation outcomes. The *in silico* evaluation identified two structural alerts in the cleansing conditioner and six structural alerts in the hair dye ingredients. When tested *in vitro*, the cleansing conditioner alone did not cause an irritation response. The hair dye alone caused a reduction in tissue viability, but was not sufficient to be classified as an irritant. Interestingly, when tested in combination as a 50/50 mixture, the reduction in tissue viability was approximately half of the response observed with hair dye alone. The results of this study showed: 1) not all the hazards identified *in silico* led to an *in vitro* response; 2) treatment with cleansing conditioner alone did not elicit a response while treatment with hair dye alone reduced tissue viability that may lead to irritation at sufficient exposures; and 3) the two test articles did not interact synergistically. However, it is important to note that this study was unable to evaluate the undiluted effects when articles were tested in combination. Further development is necessary to truly evaluate aggregate or cumulative exposures from multi-product use.

2383 Targeted Protein Degraders: Considerations for Nonclinical Safety Assessment of a New Pharmaceutical Modality

J. Kinyamu-Akunda. *Novartis Institute for BioMedical Research, East Hanover, NJ.*

Targeted/induced protein degradation has emerged as a novel drug discovery approach with promise for drugging targets previously considered undruggable. Targeted protein degradation exploits the intrinsic cellular machinery for disposing of damaged, misfolded, or excess proteins. The approach consists of low molecular weight agents that enable tagging of a protein of interest by an E3 ubiquitin ligase for subsequent ubiquitination and degradation in the proteasome. Recently, the first targeted "degrader" entered into clinical trials in prostate cancer (Mallard A. *Nature Reviews Drug Discovery* 18:237), and multiple others are in preclinical development. There are a number of advantages for degraders over traditional low molecular weight inhibitors, including prolonged pharmacodymics due to lag time for protein resynthesis and increased potency due to a potential for one molecule to repeatedly catalyze ligand activity. Moreover, a broader spectrum of activity may result from whole protein degradation compared with protein inhibition. Presumably, this should result in need for lower therapeutic exposures, better selectivity, and improved margins of safety. This session seeks to introduce the SOT community to this new area of intense research in the pharmaceutical industry and share learning from experts. The presenters aim to start a discussion that gets the toxicology community ahead of this emerging science to define important parameters to consider for characterizing the safety of this new modality. After a brief introduction to targeted protein degraders, the first speaker will discuss ADME properties and considerations for PKPD and TKTD relationships. A number of the current degraders in preclinical development are derived from iMiDs (immunomodulatory modulatory drugs) such as thalidomide and utilize the ability of the glutarimide moiety to bind cereblon and provide a chemical anchor to the E3 ligase. Thalidomide is a well-known human teratogen, and other iMiDs likewise show thalidomide-like teratogenicity in animals. Recent publications report that iMiDs degrade SALL4, a zinc finger transcription factor shown to be causative for human syndromes characterized by malformations that phenocopy thalidomide malformations (Kohlhase et al. 2003). The second speaker will discuss recent advances in understanding thalidomide teratogenicity and how this informs strategies for safety assessment of protein degraders. Finally, the third and last speaker will discuss general safety considerations for protein degraders, highlighting case examples.

2384 ADME and PKPD Considerations for Targeted Protein Degraders

D. Hainzl. *Novartis Institutes for BioMedical Research, Cambridge, MA.* Sponsor: J. Kinyamu-Akunda

Most of the degraders currently in development contain three parts: the targeted protein-binding moiety, the ligase binder, and a linker that connects the two. These structural requirements and the desire for high binding affinity and specificity make the molecules complex and large, leading to molecular properties that make development challenging. IMiD drugs that function as molecular glues may overcome some of these challenges as they combine the two binding domains without the use of a linker and thus minimize the size of the molecule. This talk will discuss the opportunities and challenges when addressing and optimizing the absorption, metabolism, distribution, and elimination (ADME) of the degraders and how this relates to considerations of PKPD and toxicity.

2385 SALL4 Degradation by Cereblon Ligands and Its Implications for Teratogenicity of Degraders

E. Fischer. *Harvard Medical School, Cambridge, MA.* Sponsor: J. Kinyamu-Akunda

Despite their teratogenicity, thalidomide and related IMiD drugs are now a mainstay of cancer treatment; however, the molecular basis underlying the pleiotropic biology and characteristic birth defects remains unknown. Recently, thalidomide and other iMiDs were shown to degrade of several C2H2 zinc finger transcription factors, including SALL4, a member of the spalt-like family of developmental transcription factors. Heterozygous loss of function mutations in SALL4 results in a human developmental condition that phenocopies thalidomide-induced birth defects such as absence of thumbs, phocomelia, defects in ear and eye development, and congenital heart disease. Thalidomide induced degradation of SALL4 in humans, primates, and rabbits, but not in rodents or fish, providing a mechanistic link for the spe-

cies-specific pathogenesis of thalidomide syndrome (Donovan et al. 2018). This presentation will focus on identification of SALL4 and the implications of these data in informing toxicology strategies for iMiD-derived protein degraders.

2386 Important Considerations for Safety Assessment of Targeted Protein Degraders

K. Datta. *Celgene Corporation, Summit, NJ.*

The safety of targeted protein degraders is primarily attributed to the pharmacologic mechanism of action. Thalidomide and other iMiDs act as molecular glue degraders that lead to degradation of cereblon-related protein substrates such as Ikaros and Aiolos, transcription factors involved in hematopoiesis. The toxicity associated with iMiDs is largely hematopoietic and likely on target; however, as mentioned previously, iMiDs are teratogenic in animals in the case of thalidomide in humans. Safety assessment of protein degraders entails conventional small molecule early safety profiling, and aspects specific to this mode of action, such as evaluation of degradation of intended protein(s) and potentially unintended proteins to minimize potential safety concerns. The speaker will present case studies highlighting the nature of species-specific toxicities in IMiD-treated animals. Additionally, effects of IMiDs on immune function, including responses to immunization, will be discussed.

2387 Communicating Risk in a (Mis)information-Rich World

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Understanding the risks and hazards consumers face has become an overwhelming task for them. Inaccurate, yet compelling, narratives and visuals promulgated via social media drown out science-based messaging and widen the gap between actual risks and risk perception, creating uncertainty and confusion for consumers. In a world where we are prone to cognitive leaps and scary stuff is always easier to believe, how can consumers make sense of complex information related to risk, especially in areas where there is uncertainty? As risk communicators, we are constantly challenged to operate in this complex environment. In line with SOT Strategic Priority to increase the Society's influence through science communication, this session brings together speakers with various perspectives to help attendees to better understand how the public recognizes and reacts to risk messaging and to find ways of proactively and effectively communicating in an information-rich environment to foster trust and critical thinking. For this panel session, our experts will (1) discuss how the media and social media shape public perceptions of science and risk; (2) showcase examples of how risk can be communicated in meaningful ways to diverse audiences, specifically as it relates to current issues in toxicology, including per- and polyfluoroalkyl substances (PFAS); (3) explore options (remedies) for empowering the public to think critically about exposures and toxicology; and (4) provide strategies and practical skills for attendees to apply in their own outreach and communication efforts to increase public awareness and understanding of risk in the context of toxicology. Each speaker will give a short talk drawing on their specific expertise and experience of the speakers in the world of science and risk communication. A wide range of topics will be covered, from the science of science communication, to how public perception of toxicology and risk is shaped, to the importance of collaboration with stakeholders in risk communication. This session also will discuss how errors in risk perception underlie much of public misunderstanding of science and how media coverage and social media algorithms skew public risk perception. Topics also will focus on the importance of collaboration between multiple stakeholders and interagency collaboration in the development and dissemination of public health messages, as well as strategies for effective communication that allow you not only to convey information, but also to engage and empower your audience to listen and respond. Talks will be followed by a moderated discussion on current issues in risk communication related to toxicology. Issues covered will include message development, strategies and practical skills for communicating complex risks in a timely manner, and accurately conveying uncertain risks when research is ongoing. Panelists will use current "hot topics" in toxicology (e.g., PFAS) to illustrate these concepts. PFAS are man-made surfactants (including PFOA, PFOS, GenX, and many other chemicals) found in a wide range of consumer products. They are persistent and bioaccumulate, and exposure can lead to adverse health outcomes in humans. The panel of science communication experts will share their knowledge, approaches, and experiences related to these important communications issues. Attendees will take away information that will help develop skills to apply in their own efforts to increase public awareness and understanding of risk in the context of toxicology.

2388 Current Status and Knowledge Gaps in the Use of Zebrafish in Risk Assessment

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Over the past five years, there has been much advancement in the use of zebrafish as a powerful alternative model in drug and toxicity screening. Extensive research has been done on understanding chemical space for which zebrafish is a good predictor in reliably prioritizing compounds for screening and in risk assessment, and cases where it cannot be used reliably (e.g., volatile compounds). This session summarizes advancements in the field with respect to similarities and differences between zebrafish and mammalian systems, presents strengths and limitations of the zebrafish in assessing safety and risk assessment to ensure reliable and good translational data, provides case examples of where the zebrafish has been a good predictor of toxicity and where it has not, and emphasizes the need for harmonization across laboratories conducting zebrafish research. It also will highlight stengths, lessons learned, and knowledge gaps in the current use of zebrafish as a complementary model in drug and toxicity screening and prioritization. The first presentation will introduce a risk assessment screening strategy used to identify candidates with developmental, and neurodevelopmental (DNT) or neurotoxicity, cardiotoxicity and hepatic toxicity utilizing a tiered screening approach. Utilizing a tiered screening approach, candidates will be able to be classified as cardiotoxic, hepatotoxic, developmental or neurodevelopmental and neurotoxic, after a funnel of toxicity assays designed based on previous validation and standardization data. Getting risk assessment data that can help in chemical prioritization and also translational to other animal models (like mammals), ending in safer candidates. The second speaker will present on a multidimensional zebrafish assay system to describe the systematic screening of flame retardant chemicals (FRCs), enhancing the protocol design's importance to get reliable and transferable data to other labs developing the same assays. The third presentation will highlight how developmental neurotoxicity assays coupled with analytical chemistry can be used to identify shared toxicity phenotypes arising from structurally similar per- and polyfluoroalkyl substances (PFAS). The final presentation will provide insight into using the zebrafish as a tool to screen environmental compounds for risk assessment and how the measurement of benchmark concentrations (BMC) may be used as a common data analysis method in comparing data across laboratories. Collectively, this session will capture the latest advancements in the design, execution, and analysis of zebrafish toxicity data to enable chemical prioritization and risk assessment with more efficiency.

2389 A CURE for the Common Classroom: Engaging Students and Faculty in Course-Based Undergraduate Research Experiences to Maximize Academic Success and Productivity

L. Williams. *Bates College, Lewiston, ME.*

Research in science is approached through asking novel questions with unknown answers. However, the way in which science has been historically taught at the undergraduate level is through cookbook labs with known and predictable methods and answers. Core competencies recommended by the 2011 report of the American Association for the Advancement of Science and National Science Foundation—*Vision and Change in Undergraduate Biology Education: A Call to Action*—have pushed educators to teach science in an authentic manner and expand research opportunities to a greater number of students. One model that has been widely adopted to meet these goals is that of Course-based Undergraduate Research Experiences (CURE). These courses, the research experience and cultivation of science identity of which can be incorporated into new or existing courses, can span from the first year of study to the upper level. While these courses take a variety of forms, national assessment data have shown that CUREs increase content knowledge, analytical and technical skills, and persistence in science for all students, but especially for students underrepresented in STEM. In addition to student benefits of this model, faculty report increases in their own research productivity. In this session, we will report CURE models from the first year of study to the upper level at both large research institutions and small undergraduate-only institutions and present the benefits of this model to students and faculty alike.

2390 Environmental Carcinogenesis: Effect of Carcinogen Exposure on the Genome and Health Risks

I. Pogribny. *US FDA/NCTR, Jefferson, AR.*

Carcinogenesis in humans is a dynamic and complex phenomenon determined by the interface between two major etiological factors, genetics and environmental carcinogens, among which the exposure to natural and man-

made chemical and physical carcinogenic agents has a more profound impact on cancer development. Currently, it is well recognized that the most impactful approach to reduce the burden of cancer is the early detection and precision prevention strategy, which focuses on individuals who have not been diagnosed with cancer. The success of this approach deeply relies on a better understanding of the fundamental biology of early carcinogenesis, especially biomarkers of exposure and molecular drivers of preneoplasia that may not only define etiologic risk but also delineate interindividual heterogenicity in response to carcinogenic insults and cancer development. This Symposium will present current knowledge on the role of environmental chemicals in the cause of cancer and how environmental insults are involved in the carcinogenic process and illustrate the linkage between exposure and genome alterations as a central component of environmental carcinogenesis. Additionally, it will highlight new conceptual and technological advances in the field of carcinogenesis, outlining the importance of the evaluation of carcinogen-induced genome alterations not only in the carcinogen assessment process but also in the development of precise cancer preventive strategies.

S 2391 Understanding the Mechanisms of Chemically Induced Carcinogenesis in Application to Potential Human Risk

J. E. Klaunig. *Indiana University, Bloomington, IN.*

The mechanisms by which environmental agents induce cancer are important in developing meaningful, scientifically based human risk assessment. Cancer is a multistage process involving target cell mutation changes and selective proliferation of this mutated cell to a neoplasm. In rodents, the liver is a frequent target of these chemicals. Using the rodent liver model, the mechanisms of chemically induced carcinogenesis have been extensively studied and can be divided into genotoxic and nongenotoxic modes of action. Application of newer cellular and molecular techniques, including 'omic approaches, have contributed greatly to this understanding. The genotoxic mode of action involves interaction and damage to genomic DNA, while nongenotoxic mechanisms involve modification of gene expression—particularly those genes involved in growth control, metabolism, inflammation, and oxidative stress. In the case of the latter, excess reactive oxygen can result in damage to and modification of cellular macromolecules, most importantly genomic DNA that can produce mutations as well as modulate gene expression of downstream targets involved in DNA repair, cell proliferation, and antioxidants. In the liver this occurs through receptor-mediated processes (frequently involving CAR, PXR, PPAR-alpha, or AhR) or via cytotoxicity. For example, the induction of rodent hepatic tumors has been seen for a diverse and large group of chemicals that function through PPAR-alpha activation, including DEHP, PFOA, TCE, and clofibrate. While PPAR-alpha activation in the rodent liver results in changes in cancer-related gene expression including increases cell proliferation, similar cellular effects have not been observed in humans. Thus, while the cellular and molecular underpinning of the mechanisms involved in rodent liver cancer is similar in humans, dose-response and species differences in gene activation and the resulting apical endpoints important in the carcinogenesis process are seen that need to be considered when examining human cancer relevance to environmental chemicals.

S 2392 DNA Adductomics Technologies for Assessing Human Exposures to Carcinogens: Potentials and Challenges

R. J. Turesky, J. Guo, S. Walmsley, B. Yun, P. Murugan, C. J. Weight, and P. W. Villalta. *University of Minnesota, Minneapolis, MN.* Sponsor: I. Pogribny

Humans are frequently exposed to low levels of a wide mixture of environmental and dietary genotoxicants, and endogenously produced electrophiles. These reactive species can damage DNA and form covalent modifications, known as adducts. DNA adducts formed at critical sites in tumor-related genes are believed to be the first step in chemical carcinogenesis. Historically, DNA adducts have been measured by targeted methods where one up to several adducts are monitored in an assay. However, the data often provide limited evidence for a role of a chemical in the etiology of human cancer. There is a critical need to develop "untargeted" methods that simultaneously detect many DNA adducts in the genome; some of which may induce mutations and lead to the onset of cancer. With the recent improvements in the sensitivity and scanning rates of high-resolution mass spectrometry (MS) instruments, such as quadrupole time-of-flight and hybrid Orbitrap MS, it is now feasible to screen for an array of DNA damage in the human genome using adductomics approaches. The loss of 2'-deoxyribose from the modified nucleoside upon collision-induced dissociation is a characteristic feature and the most commonly used transition in the screening of DNA adducts by targeted and nontargeted MS methods. We have adapted several advanced data-dependent (DDA) and data-independent acquisition (DIA) scanning techniques originated from proteomics and metabolomics and tailored them for DNA adductomics. These scanning methods have identified DNA adducts of carcinogens formed in tobacco smoke, cooked meats, traditional herbal medicines, and endogenously produced electrophiles in human bladder, renal cortex, and prostate tissues. DNA adductomics is a new and developing technology for human exposure assessment. As the analytical technology matures and bioinformatics tools become available for automated analysis of the mass spectral data, DNA adductomics can advance our understanding of the role chemical exposures play in DNA damage and cancer risk.

S 2393 Mutational Signatures of Carcinogen Exposures and Their Functional Impact

J. Zavadil. *IARC, Lyon, France.* Sponsor: I. Pogribny

The International Agency for Research on Cancer (IARC) aims to identify preventable cancer causes; to this end, the ongoing research programs employ innovative experimental models and computational approaches to investigate previously unrecognized mutagenic effects of human carcinogens. The selection of the studied compounds primarily reflects the priorities set for carcinogen evaluation by IARC. The effects of candidate substances are studied in clonally expanded cell lines that undergo biological barrier bypass to mimic early steps of cell transformation, and by massively parallel sequencing conducted at the genome scale. Complementary *in vitro* adductomics experiments as well as sequencing of rodent tumors arising from corresponding chemical exposures further elucidate the underlying mutagenic processes. The experimental results are matched with the sequencing data from human pan-cancer genome repositories, to identify relevant exposure fingerprints in humans. Several novel mutational signatures associated with dietary compounds, mycotoxins, therapeutic agents, and industrial chemicals will be presented, alongside mechanistic insights into the possible causes of particular cancer types. Furthermore, our cell models allow for comprehensive analysis of the exposure impact on the epigenome (e.g., chromatin structure/accessibility) and on gene expression regulation, in the context of carcinogen-induced cellular immortalization. Overall, this powerful approach has a considerable potential for improved understanding of extrinsic cancer causes and for cancer prevention efforts aimed at modifiable exposures.

S 2394 Use of Gene Expression Profiles to Facilitate Read-Across of Per- and Polyfluoroalkylated Substances in Human Primary Liver Cell Spheroids

E. Atlas, A. Rowan-Carroll, K. Leingartner, B. Kuo, R. Gagne, J. Bourdon-Lacombe, S. Labib, S. Ferguson, and C. Yauk. *Health Canada, Ottawa, Canada.* Sponsor: I. Pogribny

Per- and poly-fluoroalkylated substances (PFAS) are a class of chemicals ubiquitously found in the environment due to their wide variety of uses, their persistence, and their high mobility. Concerns for potential adverse health effects include liver and kidney toxicity, increased cholesterol levels, and effects on lipid metabolism. There is a growing body of evidence showing that PFOS (perfluorooctanesulfonic acid) and PFOA (perfluorooctanoic acid) are toxic and bioaccumulative; however, there is little available information on the many other PFAS found in the environment and humans. Acquiring information on data-poor substances for risk assessment has been a challenge for Health Canada and other regulatory agencies given the cost and length of traditional toxicological research. The goal of this project is to evaluate the biological perturbations induced by 20 prioritized PFAS and seven mixtures to explore mode of action/mechanistic similarities and compare potencies to inform read-across. One of the major target organs for PFOS and PFOA toxicity in rodent studies is the liver, where they are suspected carcinogens. Therefore, we employed gene expression profiling in human primary liver cell spheroids in response to PFAS using TempO-seq to identify biological effects in a concentration-response and time-series design (four time points). In parallel, we used microscopy to evaluate phenotypic changes related to liver pathology. We initiated the study using four prototype PFAS (PFOS, PFOA, PFDS, PFBS). Identification of differentially expressed genes (DEGs), hierarchical clustering, pathway analysis, and benchmark dose (BMD) analysis was conducted. PFOS was the most biologically active (most DEGs) and had the lowest gene and pathway BMDs, indicating that PFOS is the most potent of these PFAS. By day 14, however, the BMDs for PFOA and PFDS were highly overlapping with PFOS. In contrast, PFBS consistently had the fewest DEGs and highest median BMD for genes and pathways for all time points. Analysis of the affected pathways, in response to PFAS, showed effects on lipid metabolism and identified PPARα as an upstream regulator for all four PFAS. The data thus far indicate that these chemicals have similar toxicity in the liver cell spheroids and may have a common molecular target (PPARα), but their potencies differ.

2395 Use of Epigenomic Markers of Exposure in Carcinogen Detection *In Vivo* and *In Vitro*

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Human exposure to certain natural and man-made chemical carcinogens is one of the major risk factors for cancer development. In a broad sense, the carcinogenic process may be induced through either genotoxic (agents that interact with DNA) or nongenotoxic (chemicals causing tumor formation by mechanisms other than directly damaging DNA) carcinogens. Currently, the identification of carcinogen-induced genetic changes such as DNA or protein adducts often is a first step in elucidating the potential role of a genotoxic chemical in the etiology of human cancer, and it is widely accepted that detection of DNA or protein adducts caused by exposure is an endpoint indicator in cancer risk assessment. In contrast, there are no regulatory accepted short- or medium-term tests that can predict nongenotoxic carcinogenicity. Through examples of epigenetic alterations caused by the exposure to model genotoxic (aflatoxin B1, benzo[a]pyrene, and acrylamide) and nongenotoxic (arsenic, furan, and methapyrilene) chemicals, this presentation will highlight the role of epigenetic events as the earliest markers of exposure and their role in carcinogen detection *in vivo* and *in vitro*.

2396 Epigenetic Mechanisms of Metal-Induced Cancer Stem Cell-Like Property and Carcinogenesis

C. Yang. *University of Kentucky, Lexington, KY.*

Several common metal carcinogens, including arsenic (As), cadmium (Cd), and nickel (Ni), are weakly mutagenic. This suggests that nongenotoxic mechanisms, such as epigenetic mechanisms, may play critical roles in carcinogenesis induced by these metals. Although generally considered a genotoxic carcinogen, studies show that Cr(VI) exposure also can trigger epigenetic alterations. This 165-minute Symposium convenes a panel of outstanding researchers in cancer epigenetics and metal carcinogenesis fields to discuss the critical roles of epigenetic deregulations in metal-induced cancer stem cell-like properties and carcinogenesis. The first presenter, an expert in cancer epigenetics research, will discuss epigenetic deregulation in cancer stemness, carcinogenesis, and cancer therapy. Next, four experts in the metals carcinogenesis research field will present their cutting-edge studies to show how epigenetic deregulation is critically involved in metal-induced cancer stem cell-like properties and carcinogenesis. This session differs from and expands on the previous year's metal carcinogenesis Symposium by (1) presenting research that discusses epigenetic mechanisms of four different metals (As, Cd, Ni, Cr), thereby reaching a wider audience of metal researchers; (2) including epigenetic mechanisms of metal-induced cancer stem cell-like properties and their role in metal carcinogenesis; and (3) including a presentation by a cancer epigenetics expert who will talk about the background of epigenetics and how epigenetic mechanisms are key in carcinogenesis and cancer therapy. Discussions about the knowledge gaps in epigenetics in cancer and therapies would appeal to many researchers, not just in metals and cancer, but in epigenetics in general. This Symposium session will be of interest to scientists involved in metals, stem cells/cancer stem cells, or cancer research and will be ideal for those who desire a better understanding of the epigenetic mechanisms involved in metals carcinogenesis.

2397 Environmental Exposures, Carcinogenesis, and Reprogramming of the Cancer Epigenome

J. Ohm. *Roswell Park Comprehensive Cancer Center, Buffalo, NY.* Sponsor: C. Yang

In disease, the term *epigenetic* refers to heritable changes in gene expression that are not caused by underlying mutations. Epigenetic mechanisms influence and regulate how the tumor interacts with its microenvironment, the immune landscape of cancer, and metabolic changes associated with tumorigenesis. Abnormal epigenetic reprogramming is a hallmark of all types of cancer, and unlike genetic changes in a cell, epigenetic changes are potentially reversible with targeted therapeutic strategies. Epigenetic drugs include DNA hypomethylating agents, histone deacetylace (HDAC) inhibitors, micro-RNA targeted therapies, and small molecule inhibitors of epigenetic modifying proteins, and many of these drugs are already being tested for some cancers, creating unique opportunities for therapeutic intervention. This presentation will provide an overview about epigenetics, epigenetics and carcinogenesis, and cancer therapy. Epigenetic regulation of cancer stemness and how environmental exposure deregulates epigenetics and promotes carcinogenesis will be highlighted. While our understanding of the reprogramming of the cancer epigenome has increased exponentially over the last two decades, there are still huge gaps in our knowledge of the genetic and environmental factors responsible for the initiation of epigenetic remodeling in cancer. Increasing our understanding of epigenetic mechanisms of environmental carcinogenesis is essential to developing appropriate targeted therapies and prevention strategies for those with environmental exposures.

2398 Metabolomic and Epigenetic Signatures of the Arsenic-Induced Cancer Stem Cells

F. Chen. *Wayne State University, Detroit, MI.*

Exposure to environmental arsenic, especially trivalent inorganic arsenic (As3+), either from natural drinking water contamination, working place air pollution, using of certain types of pesticides, or consuming As3+-enriched food, has been linked to human cancers. However, the mechanism of arsenic carcinogenesis has not been well understood. Cancer stem cells are considered as cancer initiation cells playing important role in carcinogenesis. This presentation will first briefly discuss cancer stem cells and will then present studies showing that arsenic exposure produces cancer stem cell-like cells. By mimicking the human exposure using the environmentally relevant concentration of As3+ for a six-month consecutive treatment of the human bronchial epithelial cells, we not only observed malignant transformation of the treated cells but also noted that some of the transformed cells acquired characteristics of cancer stem-like cells (CSCs). Moreover, integrated transcriptomic and untargeted metabolomics analyses demonstrated a higher rate of glycolysis in CSCs. Unlike cancer cells and embryonic stem cells (ESCs) that use glycolysis for lactate production, the majority of the glycolytic intermediates in CSCs shunted into the siding pathways of glycolysis for hexosamine biosynthesis associated with protein O-GlcNAcylation, and the serine/glycine pathway linked to the generation of S-adenosylmethionine (SAM). ChIP-seq data revealed an enhanced enrichment of histone H3 lysine4 trimethylation (H3K4me3), an active epigenetic marker for gene transcription, among genes important for stemness and glycolysis. Additional biochemical analysis suggested that the metabolomics as well as epigenetic features of the arsenic-induced CSCs are partially dependent on the activation of Nrf2 and its downstream signaling.

2399 Epigenetic Effects of Cadmium in Carcinogenesis

E. J. Tokar. *NIEHS, Research Triangle Park, NC.*

Cadmium has been a well-established human carcinogen for decades; however, its mechanisms of action are not fully defined. Studies indicate that cadmium is, at most, weakly mutagenic in human cells, which suggests that carcinogenesis induced by this metal is mediated through epigenetic mechanisms. The first part of this presentation will review the epigenetic effects induced by cadmium that result in the acquisition of multiple hallmarks of cancer and malignant transformation. DNA methylation induced by cadmium can occur globally (hyper-/hypomethylation), in an agglomerative fashion (large chromosomal regions), or via methylation of specific genes known to regulate carcinogenesis. The dependence of these epigenetic effects on the duration of exposure, cell type used, and/or DNA methyltransferase activity will be described. Cadmium also can increase global methylation of histones (e.g., H3K4me3 and H3K9me2) by inhibiting the activities of their respective demethylases. These modifications alter the expression of cancer-associated genes or non-coding RNAs, appear to occur early in the carcinogenic process, and affect the expression of genes that regulate stem cells (SCs). The aberrant alteration of various miRNAs by cadmium can facilitate carcinogenesis and "recruit" SCs into a cancer SC-like phenotype. The presentation will end with a discussion of emerging evidence that suggests early-life (i.e., prenatal) exposures to cadmium can lead to long-term epigenetic dysregulation. While much of this evidence is suggestive in the context of cancer, it is compelling that many of the effects occur in SC- and/or cancer-associated genes and signaling pathways. The implications of these changes for possible later-life disease manifestation will be described.

2400 Nickel Exposure Causes Persistent Transcriptional Changes through Deregulation of the Epigenome

S. Cuddapah. *New York University, New York, NY.*

Nickel (Ni) compounds are environmental carcinogens associated with lung and nasal cancers in humans. However, the molecular basis of Ni carcinogenicity is not fully understood. Accumulating evidence suggests that epigenetic and transcriptional deregulations contribute to Ni carcinogenesis.

This talk will briefly present on the transcriptome and epigenome. Then, findings about how Ni exposure deregulates transcriptome and epigenome will be presented. To gain mechanistic insights into Ni exposure-caused cancer and other diseases, we comprehensively analyzed the transcriptome and the epigenome of Ni-exposed human lung epithelial cells. Our studies showed that the Ni exposure-induced transcriptional alterations persisted even after the cessation of exposure. By examining the persistent effects of Ni exposure, we show that Ni induces epithelial-mesenchymal transition (EMT) and that the mesenchymal phenotype remains irreversible even after the termination of exposure. Examination of the epigenetic features of Ni-exposed cells revealed genome-wide alterations to the histone modification profiles, which persisted long after the termination of exposure. Our results suggest stable alterations to the epigenomic landscape as the basis for long-term gene expression changes and EMT in Ni-exposed cells. Activation of EMT, during which the epithelial cells lose cell-cell adhesion and become migratory and invasive, plays an important role in the etiology of several lung diseases associated with Ni exposure, including asthma, pulmonary fibrosis, and cancer and metastasis. Therefore, our finding of long-term epigenetic changes due to Ni exposure and the acquisition of persistent mesenchymal phenotype have major implications in understanding Ni-induced cancer and other diseases.

S 2401 Epigenetic Mechanism of Chronic Hexavalent Chromium Exposure-Induced Cancer Stem Cell-Like Property and Tumorigenesis

Z. Wang. *University of Kentucky, Lexington, KY.*

Although hexavalent chromium (Cr[VI]) is a well-known and common environmental and occupational carcinogen, the mechanism of Cr(VI) carcinogenesis has not been clearly defined. While Cr(VI) is generally considered as a genotoxic carcinogen, studies showed that Cr(VI) exposure also causes nongenotoxic effects such as epigenetic changes. However, how epigenetics deregulation promotes Cr(VI) carcinogenesis remains largely unknown. This presentation will first briefly review the reported epigenetic changes caused by Cr(VI) exposure. Then, our studies about chronic low-dose Cr(VI) exposure-caused epigenetic changes and their role in Cr(VI)-induced cancer stem cell-like property and tumorigenesis will be discussed. We found that chronic low-dose Cr(VI) exposure caused epigenetic deregulations as evidenced by the increased levels of histone H3 repressive methylation marks (H3K9me2 and H3K27me3) and the related histone-lysing methyltransferases (HMTases). Knockdown of HMTases in immortalized human bronchial epithelial cells significantly reduced chronic low dose of Cr(VI) exposure-induced cancer stem cell (CSC)-like property and cell transformation. Mechanistic studies revealed that increased HMTase expression by chronic Cr(VI) exposure caused downregulation of the expression of microRNA-494 (miR-494). By bioinformatics analysis and literature searching, we found that the proto oncogene c-Myc is a target of miR-494. Western blot analysis revealed that c-Myc expression level is significantly increased in Cr(VI)-transformed cells. Stably knockeddown c-Myc expression in Cr(VI)-transformed cells significantly reduced their CSC-like property and tumorigenicity. Together, these findings suggest that Cr(VI)-caused epigenetic deregulation contributes significantly to Cr(VI)-induced CSC-like property and tumorigenesis.

S 2402 Immune Cell Polarization in Toxicology and Therapeutic Approaches

C. Rockwell. *Michigan State University, East Lansing, MI.*

Standard toxicity testing of the immune system does not currently include assays to evaluate immune cell polarization. However, there is considerable evidence to demonstrate that inappropriate immune cell polarization can result in disease. Whereas polarization of T cells into functionally distinct subsets is necessary for developing a targeted immune response that is tailored for a specific pathogen, inappropriate T cell polarization is causative in a number of diseases, including multiple sclerosis, psoriasis, asthma, allergy, and others. Likewise, macrophage phenotype switching is critical to tissue repair following injury. In addition, numerous studies have demonstrated that inflammatory macrophage phenotypes are associated with a number of different diseases. From a toxicological perspective, disruption of immune cell polarization by environmental toxicants has been well documented and can have wide-ranging functional consequences. Conversely, therapeutics that target immune cell polarization are clinically useful to restore immune homeostasis to treat immune-mediated diseases. This session will explore toxicity due to dysregulated immune polarization as well as therapeutic options for treating diseases that result from inappropriate T cell differentiation. The session aims to explore the breadth of xenobiotic effects on immune polarization resulting in different sequelae. The presenters will discuss how the industrial solvent TCE drives autoimmunity in a two-hit model by promoting Th1 and Th17 differentiation, and conversely demonstrate that activation of the xenobiotic sensor Nrf2 promotes Th2 differentiation and inhibits Th1 differentiation, resulting in exacerbated food allergy and suppressed host defense to influenza A. Taking a different perspective, the third presentation will provide some balance by showing how inhibition of ROR-gamma-t, the master regulator of Th17 differentiation, with a novel orally available drug is clinically useful in the treatment of collagen-induced arthritis and EAE, a mouse model of multiple sclerosis. The following presentation will expand the conversation to toxicant effects on Tfh, follicular helper T cells, and Th1 cells to demonstrate how inhibition of Tfh and Th1 differentiation by certain AhR ligands results in deficient CD8 and IgG response to influenza A infection. The final presenter will round out the session by demonstrating how acetaminophen toxicity results in defective M1/M2 macrophage switching—specifically, how M1/M2 switching is critical to repair and ultimately survival in acetaminophen-induced acute liver failure. The session also is designed to introduce different mechanisms by which toxicants dysregulate immune cell polarization.

S 2403 Epigenetic Regulation of CD4+ T Cell Polarization in a Model of Toxicant-Induced Autoimmunity

S. Blossom. *University of Arkansas for Medical Sciences, Little Rock, AR.*

Trichloroethylene (TCE) is an industrial solvent and a widespread water pollutant associated with CD4+ T cell-mediated hypersensitivity and autoimmune diseases in humans. Autoimmune-prone MRL+/+ mice after chronic TCE exposure develop an accelerated autoimmune disease associated with an increase in IFN-γ and IL-17-secreting effector CD4+ T cells. Our goal is to understand how TCE promotes the generation of these subsets to determine how these cells contribute to autoimmunity. Using whole-genome methylomics approaches in mouse effector/memory CD4+ T cells, TCE promoted hypermethylation of CpG regions that bind to polycomb group (PcG) proteins. One such PcG protein includes EZH2 that methylates histone H3 at lysine 27. Trimethylated histone H3K27 (H3K27me3) is a PcG-specific chromatin modification thought to play an important role *in trans*criptional repression. In T cells, EZH2 is highly expressed and important in regulating Treg function and effector cell differentiation and function. Based on our methylation results, we hypothesized that TCE alters EZH2 function, leading to the phenotype observed in our model. CD4+ T cell differentiation events are time dependent and difficult to elucidate *in vivo* in part due to the short half-life of effector CD4 T cells *in vivo*. One of the major oxidative metabolites of TCE, trichloroacetaldehyde hydrate (TCAH), was used as a TCE surrogate *in vitro* to test how the chemical disrupts cell differentiation processes in effector subsets that include drivers (Th1 and Th17) or protectors (Th2 and Tregs) of autoimmunity. Naïve CD4 T cells, when polarized *in vitro* in the presence of TCAH, altered many subset-specific genes associated with an autoimmune phenotype. EZH2-specific inhibitors decreased H3K27 trimethylation and altered the ability of TCAH to modulate Th1 and Treg phenotypes. These results will not only provide novel information but also underscore the potential utility of using *in vitro* differentiation assays as screening tools for chemicals that may promote hypersensitivity/autoimmunity to inform human risk assessment where current methodology is limited.

S 2404 Nrf2 Regulates Multiple Facets of T Cell Differentiation, Impacting Food Allergy and Host Defense

C. Rockwell, Y. Jin, R. A. Freeborn, and R. C. Kennedy. *Michigan State University, East Lansing, MI.*

Nrf2 is a transcription factor that is activated by numerous xenobiotics, including the food additive tBHQ. Our previous studies showed that Nrf2 promotes Th2 differentiation while suppressing Th1 differentiation. Our current studies indicate that Nrf2 directly upregulates gata3, the master regulator of Th2 differentiation. Our data show that Nrf2 binds two putative binding sites (antioxidant response elements, AREs) in the gata3 gene, as determined by DNA-binding ELISA and ChIP assay. These sites are transcriptionally active as shown by reporter gene assay. In addition to direct gene regulation, we also found that Nrf2 regulates a number of genes involved in T cell metabolism, suggesting a secondary mechanism by which Nrf2 regulates T cell differentiation. Th2 cells are known to be causative in allergy; we found that the increase in Th2 polarization by tBHQ *in vitro* correlates with worsened food allergy *in vivo*. Specifically, we found that tBHQ increases total and antigen-specific IgE and IgG1 during sensitization and exacerbates clinical symptoms of anaphylaxis, including a marked drop in body temperature following challenge. These effects correlate with increased mast cell degranulation and higher plasma concentrations of mMCP-1, as well as increased numbers and activity of Th2 cells. In contrast to Th2 cells, Th1 cells play an important role in host defense to intracellular pathogens. Our data show that the inhibition of Th1 differentiation by Nrf2 correlates with impaired host defense to influenza. We

found that mice on a tBHQ diet had increased lung inflammation as determined by histopathology, but diminished CD4 and CD8 T cell responses to primary influenza infection, which correlated with impaired viral clearance. Furthermore, we found that immunological memory was impaired by tBHQ, which caused a significantly greater drop in body weight following secondary exposure to influenza virus. Our most recent data indicate that Nrf2 also regulates Th17 differentiation, as evidenced by markedly higher production of Th17 cytokines by Nrf2-deficient CD4 T cells, suggesting that Nrf2 also may impair host defense against extracellular pathogens. Collectively, our studies demonstrate that the xenobiotic sensor Nrf2 regulates multiple facets of T cell differentiation and thus may represent a common pathway by which multiple xenobiotics may modulate immunity.

S 2405 RTA 1701 Is a Selective RORγt Inhibitor That Suppresses IL-17A Production and Is Efficacious in Mouse Models of Autoimmune Disease

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Th17 cells are a subset of IL-17A-producing CD4+ helper T cells that play a key role in autoimmune disorders. The nuclear receptor retinoic acid-related orphan receptor γt (RORγt) transcriptionally regulates the production of IL-17A and orchestrates the differentiation of Th17 cells. RTA 1701 is an orally bioavailable, selective RORγt inhibitor currently in clinical development. *In vitro*, RTA 1701 selectively inhibits the transcriptional activity of RORγt and does not inhibit the activity of the closely related transcription factors RORa and RORß. By inhibiting RORγt, RTA 1701 blocks Th17 differentiation of naïve CD4+ T cells and inhibits IL-17A production in both Th17-polarized CD4+ T cells and activated peripheral blood mononuclear cells (PBMCs) isolated from patients with psoriasis or rheumatoid arthritis. RTA 1701 treatment also reduces the expression of other RORγt target genes and Th17 signature genes (e.g., IL-17F, IL-21, CCL20) in Th17-polarized naïve CD4+ T cells. RTA 1701 exhibits oral bioavailability in rodents and cynomolgus monkeys, with dose-dependent increases in exposure over a wide dose range. In monkeys, oral administration of RTA 1701 suppresses *ex vivo* stimulation of IL-17A secretion in whole blood after single and repeat dosing. In animal models of autoimmune disease, including the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis and the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis, RTA 1701 attenuated disease symptoms. Together, these results suggest that RTA 1701 may have therapeutic potential in Th17-associated autoimmune disorders.

S 2406 The Aryl Hydrocarbon Receptor Regulates T Cell Differentiation

B. Lawrence. *University of Rochester Medical Center, Rochester, NY.*

Initially discovered because it mediates effects of toxicants from the environment, the aryl hydrocarbon receptor (AHR) also is now a fascinating potential target that could be used to modulate the immune system to treat disease. Exposure to AHR ligands modifies T cell differentiation and T cell-dependent immune responses; however, the consequences of different ligands differ across studies. To address this, we compared immunomodulation by AHR ligands that arise from different sources within the same *in vivo* disease model: infection of mice with human influenza A virus (IAV). Specifically, we used TCDD, a prototype exogenous AHR agonist; PCB126, a pollutant with documented human exposure; ITE (2-(1'H-indole-3'carbonyl)-thiazole-4-carboxylic acid methyl ester), a novel pharmaceutical; and FICZ (6-formylindolo[3,2-B] carbazole), a degradation product of tryptophan. During acute primary IAV infection, exposure to ITE, PCB126, and TCDD significantly reduced Th1 and Tfh cells, whereas FICZ increased their frequency. Similarly, TCDD, PCB126, and ITE, but not FICZ, reduced virus-specific IgG levels and CD8+ T cell responses. Yet, differences among ligands were not observed on all aspects of the response to IAV. For instance, virus-specific IgM levels, which are generally considered T cell-independent, were diminished by all four compounds. Moreover, in Cyp1a1-deficient mice, which metabolize AHR ligands more slowly, all compounds, including FICZ, reduced all metrics of the immune response to IAV. Conditional Ahr knockout mice, which lack Ahr in the entire immune system, revealed that all compounds require AHR within hematopoietic cells. Thus, ligand-specific differences in T cell-mediated responses to IAV likely reflect variances in quality, magnitude, and duration of AHR signaling. This indicates that binding affinity and metabolism are probably stronger predictors of immune effects than a compound's source of origin and that harnessing AHR will require finding a balance between dampening immune-mediated pathologies and maintaining sufficient host defenses against infection.

S 2407 Failed Phenotype Switching by Hepatic Macrophages Produces Persistent Inflammation in Acute Liver Failure by Acetaminophen

B. Copple, J. D. Strickland, and K. J. Roth. *Michigan State University, East Lansing, MI.* Sponsor: C. Rockwell

Acute liver failure resulting from acetaminophen toxicity remains a significant health problem with a high rate of mortality. Studies have revealed that acetaminophen-induced acute liver failure patients with the poorest outcome suffer from a systemic inflammatory response syndrome (SIRS) characterized by excessive production of immunomodulatory cytokines. Unfortunately, the cause of cytokine dysregulation in these patients remains poorly understood. Our studies have investigated the underlying cause of this in an animal model of acute liver failure. Treatment of mice with a dose of acetaminophen (e.g., 300 mg/kg), normally associated with full liver repair, increased expression of several proinflammatory cytokines, including tumor necrosis factor-a (TNF-a). Cytokine levels remained elevated at 48 hours in these mice but returned to baseline by 72 hours. In mice treated with a dose of acetaminophen (e.g., 600 mg/kg) that produces liver injury that fails to repair, cytokine levels were increased at 48 hours and remained elevated at 72 hours. This indicated a failure to terminate the inflammatory phase of wound healing, similar to what occurs in patients. Interestingly, in mice treated with 600 mg/kg acetaminophen, proinflammatory macrophages failed to traffic into necrotic lesions and thus were unable to clear dead cell debris by phagocytosis. Studies have suggested that phagocytosis of dead cells terminates cytokine production by proinflammatory macrophages. To evaluate this in acetaminophen overdose, proinflammatory monocytes/macrophages were isolated from the livers of mice treated 24 hours earlier with acetaminophen. Exposure of these cells to necrotic hepatocytes decreased expression of proinflammatory cytokines and other M1 macrophage markers and increased expression of arginase-1, an M2 marker. Collectively, these studies suggest that pharmacological stimulation of macrophage phagocytic pathways might be a novel approach to alleviate SIRS in acute liver failure patients.

S 2408 Organ to Silicon Chip: Emerging *In Vitro* and *In Silico* Tools for Evaluation of Cardiotoxicity

A. Bahinski. *GlaxoSmithKline plc, Collegeville, PA.*

There is a crucial need for new technologies that can quickly and reliably predict cardiovascular safety in humans due to drug and environmental exposure. Development of safe and effective drugs is currently hampered by the poor predictive power of existing preclinical models that often lead to failure of drug compounds late in their development, after they enter human clinical trials. Given the tremendous cost of drug development and the long timelines involved, as well as the need for evaluation of emerging environmental pollutants, the development of new technologies such as *in silico* platforms and novel *in vitro* platforms have the potential to provide more translational and human predictive models for cardiac toxicity. This Symposium will highlight innovative approaches to developing much-needed new toxicological methods for evaluation of cardiotoxicity beyond electrophysiology and arrhythmia. This Symposium brings together key experts from the fields of machine learning and artificial intelligence, microphysiological systems/organ-on-chips, and engineered cardiac tissues/precision medicine. They will highlight and discuss the advantages and challenges inherent in new innovative tools being applied toward identification and characterization of cardiotoxicity, in healthy and disease states, in pharmaceutical development and environmental safety.

S 2409 Novel Platforms to Interrogate Cardiac Physiology and Drug-Induced Changes in Contractility Using hiPSc-CM

K. Chaudhary. *GlaxoSmithKline plc, Collegeville, PA.* Sponsor: A. Bahinski

Cardiovascular safety liabilities are a leading cause of drug attrition in the pharmaceutical industry. Because contractility directly contributes to cardiac output and hemodynamics, it is important to ascertain drug-induced changes early on in pharmaceutical development. While changes in electrical repolarization can be modeled using heterologous expression systems, these substrates do not recapitulate contractile function of the heart. Primary cardiac myocytes are difficult to isolate, are prone to species-dependent differences, and are not amenable to higher-throughput drug screening strategies. In this discussion, we will focus on novel hiPSc-CM utilizing platforms to characterize cardiac contractile phenotype and drug-induced changes in contractility. Results from positive and negative inotropic agents will be presented, including clinically relevant drugs such as cardiac glycosides, L-type calcium

channel blockers, myofilament calcium sensitizers, and naturally occurring cardioactive hormones. Translation will be characterized by comparison with both *in vitro* (cellular/tissue contraction) and clinical efficacy (biomarkers of contractility). Both 2D and 3D formats will be highlighted, along with strategies for implementation to support drug development and patient safety.

S 2410 Engineering Functional Cardiac Microtissues for Human Disease Modeling and Drug Screening

M. McCain. *University of Southern California, Los Angeles, CA.* Sponsor: A. Bahinski

Myocardial tissue consists primarily of aligned cardiac myocytes that contract in synchrony to pump blood from the heart. The contractile output of myocardial tissue is dependent on many diverse factors, including cell and tissue microstructure, cell-cell coupling, intracellular calcium handling, ATP production, and the mechanical and biochemical properties of the extracellular matrix. Drugs and diseases that affect the myocardium can interfere with any number of these parameters. Thus, to broadly understand mechanisms of inherited and acquired forms of cardiac disease and to develop safe and effective therapies for the heart, we need modular platforms for engineering and functionally interrogating human cardiac tissue. This talk will describe how we combine tunable biomaterials, microfabrication techniques, and human cells (including stem cell derivatives) to engineer human cardiac microtissues with defined cellular and extracellular architecture. These microtissues are engineered on surfaces that enable measurements of multiple physiological outputs, including calcium handling, contractility, and metabolism. The form factor of these microtissues also is relatively scalable, such that they can be implemented for medium-throughput analyses to complement high-throughput screens and animal models.

S 2411 Cardiac Contractility *In Vitro*: From Patient-Specific to Air Pollution Studies

A. Grosberg. *University of California Irvine, Irvine, CA.* Sponsor: A. Bahinski

The heart's primary function to generate pressure to move blood throughout the body, via regular synchronized force generation by the myocardium, can be easily disrupted via toxic stimuli, such as harmful drugs or even air pollution, or because of an inherited genetic mutation that makes a patient more vulnerable to heart pathologies. An *in vitro* system that provides a mimic of heart contractile function can be a powerful tool to investigate patient-specific cardiac response as well as adverse effects of stimuli. In this work, we provide two examples of the "heart-on-a-chip" platform customized for (1) a patient-specific model of a Lamin A/C mutation and (2) use with multiday toxic exposure experiments. The nuclear lamina protein, Lamin A/C (LMNA), mutations can cause pathologies in multiple organs. For example, the progeria disease is associated with a mutation to the LMNA gene, and it leads to a devastating early aging and death. In contrast, other LMNA gene mutations do not cause early aging, but instead have a subtler effect, with patients presenting only with heart disease symptoms. Here, we demonstrate that a fibroblast cell line developed from a patient skin biopsy can be used to create patient-specific iPS-derived cardiomyocytes that recapitulate the pathogenic phenotype. These cardiomyocytes were engineered into tissues, and their contractile function was assessed by measuring developed stress in heart-on-a-chip devices. Cardiomyocytes derived from cells with the LMNA mutation show a clear reduction in the ability to develop stress when compared with cardiomyocytes with no mutations. Such patient-specific cardiomyocytes or primary cardiomyocytes can be easily used to construct heart-on-a-chip platforms to test a variety of acute stimuli. However, the inherent biological variability poses a challenge in testing stimuli that require multi-hour incubation times. To address this challenge, we have created a protocol to select films with a predictable change of stress as a function of time, providing a unique platform for testing pollution (or other stimuli) effects on cardiac function. The combination of these applications of the heart-on-a-chip platform can serve as a valuable set of assays to test toxicity of pollution, drugs, or other stimuli in a manner that targets specific subsets of populations that might have an inherent vulnerability.

S 2412 Using Weighted Gene Coregulation Network Analysis (WGCNA) to Decipher Temporal Dynamics in Cardiotoxicity

A. Rahman. *NIEHS/NTP, Morrisville, NC.*

Cardiotoxicity can be elicited through a variety of mechanisms, which lead to complex, temporally regulated compensatory processes at the molecular level. The sequence of molecular processes has evolutionary roots that, at a regulatory level, are analogous to highly complex agent-based models with self-organized behavior. One approach to delineating the evolved, self-organizational framework is to characterize the coordinated expression genes across a diverse set of toxicological perturbations using WGCNA. Here, we describe a cartographic exercise that uses WGCNA of the DrugMatrix heart gene expression data (1-5 days in duration, 101 test articles) from rat to reveal a map of gene-level co-expression relationships that are rooted in compensatory/adaptive processes associated with cardiotoxic stress. In total, the map reveals sets of genes tightly linked to well document biological processes such as cell cycle and ribosomal biogenesis, but also identifies sets of genes that are reflective of changes in cellularity and processes central to the differentiate functions of cardiac cells. We use the co-expression map as a base framework to explore the temporal dynamics of cardiotoxicity of a variety of prototype agents, such as anthracyclines, corticosteroids, and kinase inhibitors. The exercise reveals early agent-specific behavior that evolves into a general compensatory process that is likely intrinsic to the reparative function of the heart and is conserved across most test articles. We believe this analysis serves as a molecular-level point of reference that can be used to understand the capabilities and limitations of *in vitro* systems for modeling cardiotoxicity.

S 2413 Evaluation of *In Vitro* Structural Cardiotoxicity Using Deep Neural Networks

M. Maddah. *Dana Solutions LLC, Palo Alto, CA.* Sponsor: A. Bahinski

In vitro human cell models are emerging as promising platforms for preclinical drug safety testing. To fully leverage their potential, the development of robust and accurate assays is of the utmost importance. We present an innovative new tool, PhenoTox, which uses image-based artificial intelligence to quantify subtle and complex drug-induced structural changes that occur prior to noticeable cell damage or death. This method enables the interrogation of any cellular structure and provides a high level of sensitivity that has not previously been possible. The input to PhenoTox is a collection of microscopy images captured and grouped at multiple doses for the drugs of interest and a control set of images with only the vehicle applied. PhenoTox performs a series of 2-class deep convolutional neural network trainings on the input images and produces a set of metrics that quantify the degree of structural changes across all drug doses relative to controls. We present results for a panel of dugs with known cardiotoxicity profiles applied to human stem cell-derived cardiomyocytes, comparing structural toxicity measured by PhenoTox to the output of functional assays. Our preliminary results show that, for example, structural changes for Doxorubicin strongly correlate with decreased contraction displacement, whereas for Bortezomib they correlate with decreased relaxation duration, and there are no detected structural changes for Aspirin. We conclude by discussing the strengths, limitations, and future directions of the approach.

S 2414 TaRGET II Consortium: Elucidating Epigenetic Responses to the Environment across Target and Surrogate Tissues

D. Dolinoy. *University of Michigan School of Public Health, Ann Arbor, MI.*

It is increasingly recognized that exposures to chemicals affect health and disease not only by mutating genes, but also by modifying the epigenome, alterations to DNA that are mitotically heritable and lead to disease when deregulated. Thus, the US National Institute of Environmental Health Sciences (NIEHS) established the multi-phased Toxicant Exposures and Responses by Genomic and Epigenomic Regulators of Transcription (TaRGET) Program to identify epigenetic mechanisms that drive environmentally induced disease susceptibility. The second phase of the program, the TaRGET II Consortium, was established in 2016 and utilizes mouse models to evaluate when surrogate sources of DNA can be used in epigenetic epidemiology studies of human exposures and disease risk. Target tissues are those directly affected by environmental exposures (e.g., the brain by lead), while surrogate tissues are those that are easily accessible (e.g., blood). Epidemiology studies typically generate epigenetic data using surrogate tissues, but it is unknown whether associations with environmental factors observed in these surrogates correlate with those occurring in the tissues targeted by toxicants. Using perinatal exposure models of seven representative toxicants (lead, arsenic, air

pollution [$PM_{2.5}$], bisphenol A, tributyltin, dioxin, and di(2-ethylhexyl)phthalate), TaRGET II is generating epigenetic signature data in target and surrogate tissues and isolated cell populations at three life stages in mice. Epigenomic data include chromatin assembly via ATAC-seq, DNA methylation via whole genome bisulfite sequencing, and transcriptomics via RNA-seq. Through a series of talks discussing both individual and multiple perinatal exposures, the purpose of this Symposium is to familiarize the toxicological community with the consortium and discuss the impacts of these integrative analyses on the field of toxicoepigenetics. TaRGET II data are made publicly available and will enable further refinement of the design and analysis of human studies where target tissues are inaccessible and provide insight into epigenetic mechanisms behind the developmental origins of health and disease.

S 2415 Overview and Goals of the TaRGET II Consortium

F. L. Tyson. *NIEHS, Research Triangle Park, NC.*

Human reference epigenome maps have been generated by consortia, such as the National Institutes of Health Roadmap Epigenomics Project and the International Human Epigenome Consortium, allowing for discovery of cell type-specific epigenetic patterns and how these patterns may be altered during disease pathogenesis. However, epigenomic consortia have not previously sought to interrogate how epigenomic patterns are perturbed by environmental exposures and, in turn, influence susceptibility to environmental diseases. It is of paramount importance for the environmental health community to elucidate the mechanisms responsible for epigenome perturbation that may drive pathogenesis of chronic diseases. The TaRGET II Consortium was established to investigate conservation of perturbations in epigenomic marks across target tissues and cells (those adversely affected by environmental exposures) and surrogate tissues and cells (those that are easily accessible and reflect the environmental exposures), using mouse models of environmentally relevant exposures. Many human studies have begun to generate epigenetic data using surrogate tissues (e.g., blood). Data from TaRGET II will provide additional exposure-specific insights, and next-generation sequencing epigenetic signature data will enable further refinement of the design and analysis of human studies where target tissues are inaccessible. This presentation will provide an overview of the consortium's overarching goals and activities, taking advantage of next-generation sequencing approaches and integrative analyses.

S 2416 Integrated Analysis of Consortium-Wide Chromatin Structure and Gene Expression

B. Zhang. *Washington University in St. Louis, St. Louis, MO.* Sponsor: D. Dolinoy

Environmental toxicant exposures can alter the epigenome, including chromatin accessibility, histone modifications, and DNA methylation. The reprogrammed epigenome could lead to the dysregulated transcriptome and result in altered phenotypes and diseases, including developmental disorders and cancer. The TaRGET II Data Coordination Center (DCC) systematically integrated and analyzed 411 epigenomes and 803 transcriptomes generated by consortia members, which represented 530 liver and 305 blood samples associated with seven distinct toxicant environmental exposures, including TCDD, BPA, $PM_{2.5}$, DEHP, Pb, TBT, and arsenite, derived from mice of both sexes. We identified over 30,000 regulatory elements in the mouse genome that responded to the seven toxicant exposures and were associated with expression changes in over 3,000 genes at five months of age, approximately four months after exposure cessation. Our pan-exposure analysis suggested that DEHP and Pb only moderately affected the epigenome and transcriptome of liver and immune cells. However, BPA and arsenite dramatically reprogramed the liver and blood epigenome and resulted in significant transcriptomic changes. We further discovered strong sex-specific responses to BPA (male) and to arsenite (female). We also found air pollution ($PM_{2.5}$) significantly associated with altered epigenome and transcriptome in both liver and blood; however, different ingredients of $PM_{2.5}$ exposure resulted in distinct epigenetic and transcriptomic responses. We observed that individual animals may differ drastically in responding to the same exposure, such as TBT, underscoring individual-specific tolerance. By cross-comparing the liver and blood data, we defined the "exposure signatures" at epigenomic and transcriptomic levels for each exposure. Although most of the signatures are exposure specific, we identified a set of common signatures that were associated with multiple toxicant exposures.

S 2417 Imprinted Genes: Vulnerable Targets for Multiple Environmental Exposures?

M. Bartolomei. *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.* Sponsor: D. Dolinoy

Early-life exposures to adverse environmental stresses, including environmental chemicals, are associated with disease later in life—the so-called Developmental Origins of Health and Disease hypothesis. While the mechanisms by which these *in utero* exposures cause adverse phenotypes long after the exposure resolves remain unclear, the TaRGET II Consortium led by NIEHS is investigating the hypothesis that alterations in epigenetic gene regulation are involved. Epigenetics refers to nongenomic modifications to DNA that result in heritable changes in gene expression. Epigenetic gene regulation employs DNA methylation of cytosine residues, histone post-translational modifications, and non-coding RNAs to implement these heritable gene expression changes. One class of genes that relies on epigenetic mechanisms for their unique pattern of parental-specific expression is imprinted genes. About 200 of these genes are found in the mammalian genome—these genes are located in clusters and regulated by allele-specific DNA methylation of imprinting control regions (ICRs) for their parental-specific expression. Importantly, differential DNA methylation of ICRs must survive the genome-wide programming that occurs right after fertilization in the early mammalian embryo. Using a mouse model, we have shown that imprinting can be perturbed by a number of environmental exposures during early gestational stages, including procedures used in Assisted Reproductive Technologies and the endocrine-disrupting chemical bisphenol A (BPA). With respect to BPA within the TaRGET II Consortium, offspring from dams exposed to physiological levels of BPA through feed from two weeks prior to mating through mid-gestation exhibited disrupted DNA methylation and imprinted gene expression in a variety of imprinted genes in the placenta and embryo as well as target and surrogate tissues. This presentation will explore the hypothesis that imprinted genes are vulnerable to environmental exposure and could serve as a sensitive biomarker for epigenetic dysregulation.

S 2418 Perinatal Exposure to Lead and Phthalates Results in Altered DNA Methylation in Adult Mouse Liver and Blood: Implications for Target versus Surrogate Tissue Use in Environmental Epigenetics

L. Svoboda. *University of Michigan, Ann Arbor, MI.*

DNA methylation is a critical epigenetic mechanism linking early developmental environment to long-term health. In humans, the extent to which toxicant-induced changes in DNA methylation in surrogate tissues mirror those in the target tissues is unclear. Thus, the TaRGET II Consortium was established by NIEHS to address the utility of surrogate tissues as proxies for toxicant-induced epigenetic changes in target tissues. Using a mouse model of perinatal environmental exposures, the objective of this study was to investigate the effects of exposure to lead (Pb) or diethylhexylphthalate (DEHP) on liver and blood DNA methylation in adult male and female mice. We hypothesized that Pb and DEHP exposure would each lead to persistent changes in DNA methylation and that a subset of differentially methylated loci would overlap between liver and blood. Dams were exposed to Pb (32 ppm) in drinking water, which results in a blood lead level of 32 μg/dL, or DEHP in chow, resulting in a blood DEHP level of 144 ng/mL. Dam exposure began two weeks prior to mating and continued until offspring were weaned at three weeks of age. At five months of age, enhanced reduced-representation bisulfite sequencing was used to assess DNA methylation. Sex-stratified modeling of differential methylation by exposure was conducted using an established bioinformatics pipeline. Although Pb and DEHP exposure ceased at three weeks of age, we observed thousands of stably modified, sex-specific differentially methylated regions in the blood and liver of Pb- and DEHP-exposed animals, including nine genomically imprinted loci. In Pb-exposed males, we discovered five sites that overlapped between blood and liver and exhibited changes in DNA methylation in the same direction in both tissues. In DEHP-exposed males and females, we discovered three and two overlapping sites, respectively, with concordant changes in methylation. These data demonstrate that perinatal exposure to Pb or DEHP induces sex-specific changes in hepatic DNA methylation that persist into adulthood, some of which are consistent in the blood.

2419 Systemic Effects of Perinatal Air Pollution and Arsenic Exposure on Target and Surrogate Tissues

S. Biswal. *Johns Hopkins University, Baltimore, MD.*

Type 2 diabetes (T2D) is increasing worldwide in epidemic proportions. Both epidemiological and experimental studies indicate that environmental factors like air pollution ($PM_{2.5}$) and chronic arsenic exposure may increase the risk of insulin resistance (IR) and T2D. In particular, arsenic exposure has been shown to cause oxidative stress in trophoblastic placental tissue by producing reactive oxygen species that may cause health effects. The molecular underpinnings for these perinatal effects of exposure are mostly unknown, but reprogramming of the epigenome is involved. We hypothesize that perinatal air pollution and arsenic exposure (via maternal drinking water) results in alterations in epigenomic marks that are associated with IR, T2D, and other diseases. As a part of the NIEHS TaRGET II Consortium, C57BL/6J dams were exposed to real-world inhaled concentrated $PM_{2.5}$ (~10x ambient level/~60-120 µg/m3) or filtered air (FA) using the concentrated ambient particle system (CAPS) for 6 h/day or 10 ppb inorganic arsenite (iAs) in the drinking water, two weeks before mating until three weeks of weaning of the offspring. Even after cessation of perinatal exposure to $PM_{2.5}$, we observed persistent IR as evident from results of the glucose tolerance test (GTT) and insulin tolerance test (ITT) predominantly in males at five months of age. However, there were no significant changes in body weight due to $PM_{2.5}$. We observed that iAs exposure led to obesity in males at five months of age. In contrast, female mice prenatally exposed to iAs showed decreased in body weight. There was no significant change in level of plasma leptin in male or female mice prenatally exposed to iAs. Insulin level was decreased in female but not male in As-exposed group. We performed multi-'omic analyses (WGBS, ATAC-seq, and RNA-seq) to examine the systemic effects of exposure on target (e.g., liver) and surrogate (blood) tissue from male and female mice. This presentation will discuss an integrated analysis of these data to understand the relationship of exposure on the reprogramming of the epigenome in target and surrogate tissues.

2420 Genetic Mutations of Manganese Transporters: Clinical Presentation and Neurotoxicity

S. Mukhopadhyay. *University of Texas at Austin, Austin, TX.*

Manganese (Mn) is an essential metal, but even modest increases in brain Mn concentrations induce severe neurological effects. Historically, as early as 1837, Mn neurotoxicity has been associated with elevated exposure in occupational settings, resulting in parkinsonian-like movement disorder with dystonia. More recent studies suggest that children exposed to elevated Mn from environmental sources such as drinking water also develop neurological effects that manifest as fine motor control, emotional, cognitive, and intellectual deficits. Further, it also is now appreciated that exposure to elevated Mn during developmentally critical early periods may permanently change the trajectory of neurodevelopment and induce lifelong neurological dysfunction. Moreover, Mn is excreted in bile and feces. Patients with hepatic dysfunction, due to alcoholic cirrhosis, fail to excrete Mn, accumulate Mn in the brain, and develop neurological deficits. Overall, Mn neurotoxicity is a public health problem of global proportions. Therefore, understanding the mechanisms of Mn homeostasis and the neuropathophysiology of Mn-induced neurological dysfunction is an essential step in developing effective treatment strategies. Over the last decade, our understanding of Mn homeostasis and neurotoxicity has been revolutionized by the discoveries of three genetic disorders of Mn metabolism that induce neurotoxicity or deficiency. The first disorder was described in 2012, when homozygous mutations in SLC30A10 were reported to cause Mn-induced neurological disease. Two other genetic disorders were subsequently described—mutations in SLC39A14 also were reported to cause Mn neurotoxicity, a*nd mutations* in SLC39A8 to cause Mn and Zn deficiency. These genes code for critical metal transporters that regulate systemic uptake (SLC39A8) or excretion (SLC39A14 and SLC30A10) of Mn. Studies of the biology of these transporters and of the underlying genetic diseases are transforming our understanding of Mn homeostasis, detoxification, and neurotoxicity. This session will bring together leading experts in the field and present a cohesive overview of the current state of knowledge in the field. Five presenters will discuss innovative new discoveries from their laboratories. Dr. Mukhopadhyay discovered the function of SLC30A10 and will present on how SLC30A10 and SLC39A14 cooperatively regulate Mn levels in the body by excreting Mn. Dr. Gospe identified the first patient with mutations in SLC30A10 and will present clinical findings. Dr. Broberg identified polymorphisms in SLC30A10 and SLC39A8 in the general population and will present on epidemiological associations of these polymorphisms with risks of Mn neurotoxicity. Dr. Knutson discovered the function of SLC39A8 and will present on the unexpected role of this protein in mediating Mn uptake. Finally, Dr. Guilarte discovered changes in behavior and neuropathology in SLC39A14 knockout mice and will present on the pathobiology of Mn neurotoxicity due to this mutation. In summary, this session will encompass fundamental basic science, animal models, and human clinical studies to present a comprehensive understanding of the latest cutting-edge research in the neurobiology of Mn. The basic science presentations will comprehensively cover the similarities and differences between results obtained in models systems with observations in human patients. Content will be of interest to other scientists in the Mn and metal toxicology fields, neuroscientists, and neurologists, as well as to a broader toxicology audience interested in understanding how gene-environment interactions induce human diseases.

2421 Manganese Excretion and Neurotoxicity: Insights from Rare Genetic Diseases

S. Mukhopadhyay. *University of Texas at Austin, Austin, TX.*

Over the last few years, homozygous loss of function in SLC30A10 or SLC39A14 was reported to induce severe Mn neurotoxicity. Our recent studies revealed that SLC30A10 is a Mn efflux transporter, which, at the cellular level, transports Mn from the cytosol to the cell exterior and protects against Mn toxicity. Further, SLC39A14 primarily transports Mn into cells. Despite transporting Mn in opposite directions, at the whole organism level, these two transporters cooperatively mediate Mn excretion. Our data, obtained from full-body and tissue-specific Slc30a10 or Slc39a14 knockout mice, indicate that SLC39A14 transports Mn from blood into liver and intestines, and SLC30A10 then excretes the intracellular Mn into bile and feces. Furthermore, our recent findings revealed that brain Mn levels are primarily controlled by the excretory activity of these transporters in the digestive system. This talk will give an overview of the current state of knowledge about the inherited disorders of Mn metabolism by providing an overview of the changes seen in human patients and highlighting similarities and differences observed in animal models. Subsequently, it will focus on the excretory function of SLC30A10 and SLC39A14 and discuss the unexpected finding that the primary mode of the homeostatic control of brain Mn is excretion via the digestive system. A major highlight will be to provide the mechanism of disease due to mutations in these transporters and relate these findings to manganese neurotoxicity evident in the general population.

2422 Hereditary Disorders of Manganese Homeostasis: Natural History, Genetics, and Treatment

S. Gospe. *University of Washington, Seattle, WA.* Sponsor: T. Guilarte

Over the past two decades, three inborn errors of metabolism associated with manganese homeostasis have been described. Two of these disorders are associated with extraordinary elevations of blood levels of manganese, while the third condition is associated with a reduction in manganese levels. Patients with biallelic pathogenic variants in SLC30A10 present with early-onset progressive extrapyramidal motor dysfunction together with polycythemia and progressive liver disease, while patients with biallelic pathogenic variants in SLC39A14 have disease isolated to the central nervous system. Both disorders are associated with marked hypermanganesemia together with central nervous system manganese deposition. In contrast, patients with biallelic pathogenic variants in SLC39A8 have low or undetectable blood manganese levels and present with infantile hypotonia, failure to thrive, and intellectual disability. This lecture will review the clinical, imaging, and genetic features of these three familial disorders of manganese homeostasis. Examples of the effective chelation treatment of patients affected by the disorders of hypermanganesemia will be demonstrated. The clinical data provide the starting point for the animal- and cell-based studies that have been performed over the past few years on these proteins. Therefore, this talk will complement other presentations in this session.

2423 Manganese Transporter Genetics Influence the Susceptibility to Environmental Manganese Exposure and Neurobehavioral Outcomes in Children

K. Broberg. *Lund University, Lund, Sweden.*

Homozygous loss of function mutations in SLC30A10, SLC39A14, or SLC39A8 are rare. However, our recent studies led to the identification of common single nucleotide polymorphisms in SLC30A10 and SLC39A8 that strongly modify biological Mn concentrations in humans. Further, by a Mendelian randomization study, we have shown that Mn exposure early in life has negative effects on children's neurodevelopment, including neurobehavioral phenotypes. The polymorphisms in Mn transporters contribute to differences in sen-

sitivity to Mn exposure from the environment, where girls that are genetically less efficient at regulating Mn may be a particularly vulnerable group. The focus of this presentation will be to bring forth this emerging epidemiological data, which suggest that common variants of SLC30A10 and SLC39A8 are widely prevalent in the general population and modulate outcomes of Mn neurotoxicity. In addition to the direct human health relevance, these epidemiological studies also provide additional justification for studying the function of these critical transporters. This presentation will fit well with the description of clinical findings in patients by Dr. Gospe and the other presentations focused on the function of these transporters in cells and rodents.

W 2424 Novel Roles of ZIP8 (SLC39A8) in Manganese Homeostasis

M. Knutson. *University of Florida, Gainesville, FL.* Sponsor: S. Mukhopadhyay

Patients with loss of function mutations in the transmembrane metal-ion transport protein ZIP8 (SLC39A8) exhibit very low blood Mn levels, impaired glycosylation, skeletal abnormalities, and severe mental retardation. Studies in ZIP8-inducible knockout (ZIP8 iKO) and hepatocyte-specific ZIP8 KO mice have revealed that ZIP8 plays an essential role in Mn homeostasis by functioning in the liver, where it reclaims Mn from the bile. Accordingly, loss of ZIP8 increases biliary Mn losses, resulting in Mn deficiency. In an effort to define extrahepatic functions of ZIP8 in Mn homeostasis, we crossed ZIP8-inducible knockout (ZIP8 iKO) mice with ZIP14 knockout (Zip14-/-) mice to generate double-knockout Zip14-/-;ZIP8 iKO animals. Zip14-/- mice display impaired hepatic uptake of Mn and impaired Mn excretion, resulting in hypermanganesemia and Mn accumulation in various tissues, most notably bone, brain, and kidney. We find that while Zip14-/-;ZIP8-/- iKO mice load similar amounts of Mn in the bone and kidney as do Zip14-/- mice, they show markedly lower concentrations of brain Mn, suggesting that ZIP8 is required for brain Mn accumulation. Consistent with this hypothesis, immunofluorescence analysis of mouse brain sections indicates that ZIP8 is abundantly expressed in the choroid plexus, a principal site of Mn entry into the brain when plasma Mn concentrations are elevated. Overall, these studies have begun to define extrahepatic roles of ZIP8 in Mn homeostasis; they also identify ZIP8 as a possible therapeutic target for disorders of brain Mn accumulation. In addition to describing this data, this presentation also will relate findings in our mouse model with clinical presentations seen in human patients suffering from Mn deficiency due to loss of function mutations in ZIP8.

W 2425 SLC39A14 Knockout Mice: A Genetic Model to Study Manganese Neurotoxicity

T. R. Guilarte. *Florida International University, Miami, FL.*

Exposure to excess Mn results in parkinsonism with dystonia and cognitive function deficits. SLC39A14 is now recognized as a Mn influx transporter. Homozygous loss of function mutations of SLC39A14 result in behavioral manifestations of parkinsonism with dystonia that is not responsive to typical drugs used to treat idiopathic Parkinson's disease (iPD). The use of the SLC39A14 knockout (KO) mice provides a powerful tool to study and describe the neuropathogenesis of chronic Mn exposure along the life span. Assessment of Mn concentrations in blood and striatum of postnatal day (PN) 60 SLC39A14-KO male mice indicates a highly significant increase in Mn concentrations when compared with wild-type (WT) mice. Blood: 46.9 ± 10.7 µg/L in WT versus 975.7 ± 70.9 µg/L in KO; striatum: 2.64 ± 0.158 µg/g in WT versus 12.3 ± 1.4 µg/g in KO. Behavioral characterization of the PN60 male mice showed significant locomotor impairment, which manifests itself as a decrease in distance traveled and rearing behavior. Furthermore, preliminary data from the pole descend test indicate that there is a significant decrease in the ability to descend in KO mice, when compared with WT. Analysis of striatal dopamine (DA) concentrations and its metabolites resulted in no significant difference in the concentrations of striatal DA, DOPAC, and HVA in PN60 SLC39A14-KO male mice. Tyrosine hydroxylase immunohistochemistry in the striatum of PN140-160 animals also showed no significant differences between SLC39A14-KO mice and WT, supporting the lack of nigrostriatal dopaminergic neuron terminal degeneration in the presence of significant locomotor impairment. These findings are consistent with nonhuman primate studies from our laboratory indicating a dysfunctional but intact nigrostriatal dopaminergic system. Collectively, these results indicate that the neuropathological changes resulting from Mn overexposure are different from those observed in iPD. Our results further suggest that other neuronal systems besides dopamine are involved in Mn-induced parkinsonism with dystonia. This presentation will cover this data and relate findings to human patients suffering from Mn neurotoxicity due to mutations in SLC39A14 as well as elevated exposure to provide a comprehensive model for neurotransmitter and neurodegenerative changes that we believe underlie Mn-induced motor deficits in mammals.

W 2426 Mechanisms and Multiple Exposures: Methods to Tackle Toxicology's Most Difficult Challenges Using Systematic Review Frameworks

A. Rooney. *NIEHS/NTP, Research Triangle Park, NC.*

Systematic review approaches are increasingly being adopted for hazard, risk, and safety assessment across many disciplines in toxicology and public health. The European Food Safety Authority (EFSA), National Toxicology Program (NTP), and US Environmental Protection Agency (US EPA) have all implemented systematic review methods in the conduct of hazard assessments and in some cases risk assessments for chemicals and foods. Systematic review frameworks provide a rigorous multistep process for identifying, selecting, critically assessing, and synthesizing scientific literature for reaching evidence-based hazard conclusions on specific research questions. While systematic review methods are ideal for assessing the evidence that a specific exposure is associated with a specific health effect, application of these methods to more complex, real-world situations is more challenging. Furthermore, because the relevant data are from diverse sources and study types, application of systematic review methods to questions in toxicology and environmental health requires an approach to integrate evidence from human, animal, and mechanistic studies in reaching conclusions. This session will present approaches that have been utilized to address two principal challenges for use of systematic review methods in toxicology: (1) integrating evidence across multiple exposures, and (2) integrating evidence from mechanistic data. People are exposed to hundreds of chemicals every day, and in this context, assessing hazards from exposure to chemicals one at a time is a limiting approach. While single exposure assessments may be easier to conduct, real-world exposure to chemicals in food or consumer products or through occupational or general environmental contact involves complex mixtures that are generally at low levels. These challenges are generally recognized, and although there are efforts to address complex exposures with systematic review methods, few evaluations have been completed to date. As with the challenge presented by complex exposures, the identification, appraisal, and integration of mechanistic data is one of the most well-recognized challenges in utilization of systematic methods. There are many chemicals with little to no human or experimental animal toxicology data. *in vitro* studies that focus on mechanistic endpoints (generally upstream from phenotypic outcomes) are increasingly available and may be the only toxicology data for many chemicals. The first talk will present an evaluation of health effects from traffic-related air pollution to demonstrate how the NTP Office of Health Assessment and Translation (OHAT) Approach for Systematic Review and Evidence Integration can be used to develop hazard conclusions across multiple exposures. Subsequent presentations will highlight the utility of mechanistic data in facilitating transparent decisions, particularly in scenarios where mechanistic evidence provides clarity to findings in other evidence streams, extrapolations across species, and filling data gaps traditionally assessed by experimental animal assays. The next presentation will highlight lessons for addressing environmental health questions from the pharmaceutical industry's decade-long experience qualifying and using the mechanistic data in decision-making and predicting adverse events in the absence of human data. A strategy for evidence integration using systematic review methods that relies on mechanistic data on the estrogenicity of alkylphenols is presented in a read-across context to reach decisions on alkylphenols with little toxicity data. This will include mapping of multiple levels of data across adverse outcome pathways ranging from initiating events, such as receptor binding, to apical outcomes, such as tissue and organ changes in functionality. Finally, several case studies will be presented that highlight the role of exposure considerations within the application of systematic review approaches for the identification, assessment, and integration of mechanistic evidence in human health assessments. The case studies will illustrate integration of multiple categories of mechanistic information, including data on initiating and key events from *in vivo* and *in vitro* studies, use of ToxCast/Tox21 high-throughput studies, and synthesis of mechanistic data across multiple species, including humans. Collectively, this session will demonstrate current progress and remaining challenges in assessing real-world, complex exposures and utilizing mechanistic data in systematic assessments of hazard and risk. The presentations focus on case examples where exposure and mechanistic data challenges have been addressed. Recognizing that major challenges remain in conducting systematic reviews where the relevant studies are dominated by mechanistic data and complex exposures, the speakers will be asked to highlight principal challenges and identify potential or emerging solutions during the panel discussion to close the session.

2427 Using Systematic Review to Reach Hazard Conclusions across Multiple Exposures

B. Beverly. *NIEHS/NTP, Research Triangle Park, NC.*

The use of systematic review methods has rapidly become the gold standard to address environmental health questions due to their rigor and transparency, and these approaches are now an integral part to the decision-making process for many federal and regulatory groups. However, the adaptation of the methodology to an environmental health context is still a relatively recent occurrence, with the development of systematic review frameworks in the last five years that consider the breadth of data relevant to environmental questions. To date, few evaluations have been completed and case studies are required to test methods, refine approaches, and spur methods development. The need for case studies or guidance on how to reach hazard conclusions on multiple exposures represents a critical gap in addressing "real-world" multiple-chemical exposures, mixtures, or cumulative exposures. NTP/OHAT recently evaluated the potential effects of traffic-related air pollution on hypertensive disorders of pregnancy. The assessment of this complex exposure was developed following the OHAT Approach for Systematic Review and Evidence Integration and evaluated health effects evidence for individual components of traffic-related air pollution (e.g., particulate matter) in a stepwise manner to reach hazard conclusions. Evidence was grouped into focused exposure-outcome pairs, individual study quality was assessed, and confidence ratings were developed on each of those exposure-outcome pairs. Then, the evidence was integrated along with consideration of the independence of datasets, independence of mechanisms for the individual components, and other mechanistic data that inform the overall broader exposure of traffic-related air pollution to reach a hazard conclusion on the overall exposure. This example will illustrate how evidence can be integrated across multiple exposures using the NTP/OHAT systematic review framework and highlight the need for more case examples to further refine methods.

2428 Mechanistic Safety Tests in Decision-Making in the Absence of Human Data: Using a Systematic Review Framework

K. Tsaioun. *Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.*

Most experts agree that the current mandatory testing paradigm for prediction of effects of chemicals on human health is not always adequate to ensure public safety. Major advancements in our understanding of mechanisms of toxicity resulted in development of a number of enabling technologies that measure endpoints that correspond to the mechanistic biomarkers of human toxicity. This resulted in a number of public databases and a growing body of published literature detailing the protocols, use cases, and "fit-for-purpose" qualification results of such tests, both of which lend themselves to systematic reviews. The presentation will show an example that uses mechanistic assays in decision-making on drug candidates and an example of using a systematic review framework to find toxicological information for approved drugs in the public domain. Normalizing both datasets to human exposure using existing human data from the systematic review of literature and calculated values using some PBPK models (physiologically based pharmacokinetic models) in cases when human information is not available (e.g., environmental exposures, early drug candidates without human data) will be presented using an example of drug-induced liver injury. Application of systematic review frameworks to assess the availability and applicability of *in vitro* mechanistic tests for specific purposes in the regulatory decision-making will be detailed.

2429 Evidence Mapping and AOP-Based Integration Strategies to Support Read-Across Hypotheses in Chemical Risk Assessment: A Case Study on Estrogenicity of Alkylphenols

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Chemical interactions with the endocrine system are under increasing global scrutiny. To elicit an adverse response, endocrine-disrupting chemicals (EDCs) need to be able to perturb one or more key signaling events at the cellular level. Chemical interaction with the estrogen receptor (ER) is regarded as a key initiating event in this pathway. Among identified chemical xenoestrogens are certain alkylphenols, a group of chemicals with a wide range of industrial and consumer product applications. Among chemicals with publicly available toxicological data (i.e., the US EPA ToxCast inventory), a set of approximately 300 chemicals have been classified as alkylphenols and exhibit a continuum of ER bioactivity, suggesting that there are likely subgroups of alkylphenols that induce similar, overlapping, and possibly dissimilar biological effects. Our overall program is testing the hypothesis that the estrogenicity of alkylphenols can be predicted using an integrated computational toxicology approach combining available bioactivity screening data from the US EPA ToxCast and ToxValDB inventories, molecular docking simulations, and various data-integration techniques. This presentation will focus on the results from a comprehensive literature review and synthesis of available data to provide a map of the available evidence supporting linkages between molecular-level events (such as ER activation) and downstream adverse outcomes. Subsequent identification, categorization, and integration of mechanistic data from the peer-reviewed literature was guided by an adverse outcome pathway (AOP) framework and facilitated by an evidence-mapping approach. The utilities and challenges of software-assisted mapping tools were explored and will be discussed.

2430 Case Study Applications in the Identification, Assessment, and Integration of Mechanistic Evidence in Human Health Assessments: Consideration of Exposure in Developing Weight of Evidence Conclusions

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Mechanistic data provide valuable information regarding characterization of toxicological endpoints. Challenges in the evaluation and integration of mechanistic data are routinely described as related to heterogeneity of study designs and the lack of appraisal tools for *in vitro* studies. In recent practice of evidence-based methods, variability in construct and external validity relative to the research question and understanding of human relevance are recognized as more significant challenges. Using multiple case examples, we demonstrate the application of systematic review approaches for the identification, assessment, and integration of mechanistic evidence in human health assessments of assessing the potential for endocrine disruption and carcinogenicity (separately) and, in each, highlight the role of exposure considerations as part of problem formulation as well as appraisal and integration. By utilizing existing frameworks for organizing the evidence bases ranging from 1,000 endpoints informing potential pathways of activity based on varying complexity, including HTS data from ToxCast/Tox21 to data from observational and controlled trials in humans. In each approach, data are appraised for quality and reliability and subsequently integrated to assess the weight of evidence for each pathway or outcome using an adverse outcome pathway construct—and in some cases via quantitative methods. In addition to demonstrating various approaches for systematically integrating mechanistic data with other evidence streams, the utility and challenges in the implementation of these approaches are addressed. Possible solutions will be discussed, including consideration of exposure to help facilitate weight of evidence conclusions.

2431 Modulation of P-Glycoprotein and Breast Cancer Resistance Protein Transporter Activity by GenX (Ammonium 2,3,3,3-Tetrafluoro-2-(Heptafluoropropoxy) Propanoic Acid) *Ex Vivo* and *In Vivo* in Sprague Dawley Rats

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Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX) is a replacement for perfluorooctanoic acid (PFOA) in the production of fluoropolymers. The blood-brain barrier (BBB) protects the CNS from toxic chemicals by way of tight junctions to limit paracellular flux and active transport via the function of ATP-binding cassette (ABC) proteins. This study examined the effects of GenX (0.1 pM-1 μM) on the transport activity and expression of P-glycoprotein (P-gp), Breast Cancer Resistance Protein (BCRP), and Multidrug Resistance Associated Protein 2 (MRP2) at the BBB. *In vivo* responses to GenX were determined in Sprague Dawley rats following a single gavage dose. ABC transporter activities were determined *ex vivo* by a confocal microscopy-based assay. Western blots were used to quantify protein levels of P-gp and BCRP. Cytotoxicity was measured *in vitro* using two human cell lines co-treated with GenX (100 nM) and cytotoxic P-gp and BCRP-specific substrates. GenX exposures *in vivo* and *ex vivo* rapidly decreased P-gp and BCRP transport activity in brain capillaries from both male and female rats; however, MRP2 transport was not affected. Upon removal from the media, GenX-mediated inhibition was reversible for P-gp but not for BCRP, indicating differential reg-

ulatory mechanisms. Decreases in transport activity of P-gp and BCRP were independent of GenX acting directly on the transport proteins in cell lines that selectively expressed the individual transporters. *In vivo* dosing of GenX by oral gavage decreased P-gp and BCRP transport activity at the BBB in male and female rats. GenX exposure inhibited P-gp and BCRP transport activity *in vitro* in cultured human cells by increasing the cytotoxicity of their respective substrates. GenX significantly inhibits the activity of both brain capillary P-gp and BCRP within 1-2 h at environmentally relevant concentrations (0.1-10 nM) independent of protein expression. Because xenobiotics and endogenous molecules can affect the rate of ABC efflux transport, altered disposition of two therapeutic drugs known to be transported by P-gp and BCRP (verapamil and prazosin, respectively) following GenX exposure is being investigated. *This research was supported by the NIH Intramural Research Program [ZIA BC 011476].*

PL 2432 High Content Screening in Zebrafish Identifies Perfluorooctanesulfonamide as a Potent Developmental Toxicant

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Per- and polyfluoroalkyl substances (PFASs) have been used for decades within industrial processes and consumer products, resulting in frequent detection within the environment and human body. Using zebrafish embryos, we screened 38 PFASs for developmental toxicity at a limit concentration of 50 µM and demonstrated that perfluorooctanesulfonamide (PFOSA) was the most potent developmental toxicant, resulting in elevated mortality and developmental abnormalities following exposure from 6- 24 h post fertilization (hpf) and 6- 72 hpf. PFOSA exposures initiated during early developmental stages (0.75, 6 and 12 hpf) resulted in a concentration-dependent increase in mortality and abnormalities, with surviving embryos exhibiting up to a >12-h delay in development by 24 hpf. Exposures initiated at 0.75 hpf also resulted in a concentration-dependent delay in epiboly at 6 hpf, although these effects were not driven by a specific sensitive window of development. To identify the potential association of PFOSA-induced developmental delays with impacts on the embryonic transcriptome, we relied on mRNA-sequencing and showed that, relative to stage-matched vehicle controls, pathways related to hepatotoxicity and lipid transport were disrupted in embryos exposed to 12.5 µM PFOSA from 0.75- 14 hpf and 0.75- 24 hpf. Therefore, we measured liver area as well as neutral lipids in 128-hpf embryos exposed to vehicle (0.1% DMSO) or 0.78 µM PFOSA from 0.75 to 24 hpf and clean water from 24 to 128 hpf, and showed that PFOSA exposure resulted in a decrease in liver area and increase in yolk sac neutral lipids at a non-toxic concentration of 0.78 µM. Overall, our findings show that early exposure to PFOSA adversely impacts embryogenesis, an effect that may lead to altered lipid transport and liver development.

PL 2433 Increased Toxicity and Retention of Perflourooctane Sulfonate (PFOS) in hCYP2B6-Tg Mice Compared to Cyp2b-Null Mice Is Relieved by a High-Fat Diet

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PFOS is a flourosurfactant that has been banned in the United States, due to its toxicity. It has a half-life of about 5.5 years in humans and is associated with hepatotoxicity. Human Cytochrome P450 2B6 (CYP2B6) is expressed in liver and responsible for metabolizing xeno- and endobiotics, including polyunsaturated fatty acids (PUFAs). PFOS treatment, like high-fat diet feeding, is a potent inducer of CYP2B in human and rodent hepatocytes. Given the marked induction of CYP2B by PFOS, it was hypothesized that CYP2B is a contributor to PFOS-induced changes to liver, such as steatosis. Cyp2b-null and humanized CYP2B6 mice (hCYP2B6-Tg) were treated with PFOS (0, 1, or 10 mg/kg/day) via oral gavage with some mice receiving a typical chow diet (ND) and some receiving a 60% kCal HFD. The mice were euthanized after 21 days, and liver and serum were collected. On days 20-21, three mice died during the exposures; all three were ND-fed hCYP2B6-Tg female mice treated with 10 mg/kg/day PFOS, indicating that hCYP2B6-Tg mice were more sensitive to PFOS than the other groups. Interestingly, hCYP2B6 female mice retained significantly more serum and liver PFOS than Cyp2b-null mice. PFOS levels were typically higher in the HFD-fed mice; however, the HFD-fed hCYP2B6 females were protected from PFOS toxicity presumably due to greater white adipose tissue (WAT) and less wasting. In addition, PFOS caused a decrease in body weight due to WAT loss, and liver weights were indirectly correlated with body weight but directly correlated with PFOS concentration. Oil red O staining did not demonstrate a dose-dependent increase in liver lipid accumulation; however, PFOS in addition to a HFD exacerbated lipid accumulation. A serum and liver panel of 19 different parameters were evaluated by principle-component analysis (PCA) and indicated that PFOS severely impacted the liver with significantly elevated ALT and ALP levels demonstrating hepatotoxicity. In general, the PCA plot indicates that PFOS causes dose-dependent liver toxicity that is protected by a HFD, and that Cyp2b-null mice fed a HFD and treated with PFOS are pushed towards the cholesterol and LDH parameters, indicating a greater likelihood of cardiovascular disease. Gene expression of enzymes related to NAFLD are currently being evaluated, using a microarray, so the role of hCYP2B6 in PFOS toxicity can be better understood.

PL 2434 Per- and Polyfluoroalkyl Substance (PFAS) Concentrations in Human Placenta and Associations with Birth Outcomes

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Per- and polyfluoroalkyl substances (PFAS) are a group of widespread and persistent environmental contaminants found in drinking water, food packaging, textiles, and other consumer products. PFAS are also persistent in the human body and have been detected in human serum, breast milk, and umbilical cord blood. PFAS exposure during pregnancy has garnered concern due to the potential developmental toxicity of PFAS and known placental transfer of these compounds to the developing fetus. Prior studies have noted associations between maternal serum PFAS concentration and low birth weight; however, it is possible that those associations were influenced by changes in glomerular filtration rates (GFR) during pregnancy. To date, no studies have investigated associations between placental PFAS levels and birth outcomes, and the placenta may be a better tissue for examining fetal exposures as it would not be influenced as strongly as maternal serum by changes in GFR. To gain a better understanding of the current *in utero* PFAS exposures, human placenta samples (n=120) collected at delivery in 2010-11 from women giving birth in Durham, North Carolina were analyzed for PFAS. Archived placental samples (~1 gram) were extracted, purified, and analyzed via high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) for a suite of 11 different PFAS analytes, including the analytes PFOS, PFOA, and GenX. Furthermore, using available metadata collected at birth (e.g., infant sex, birth weight, and gestational age), we examined whether PFAS concentrations in the placenta differed by infant sex or if levels were associated with birth weight or gestational age. Analyses were adjusted for maternal smoking, race, maternal education, parity, and maternal age. PFAS were detected in all placenta samples, illustrating the prevalence of PFAS exposure. The most abundant PFAS in the placenta were the long-chain PFAS, specifically PFOS, PFOA, PFNA, and PFDA, with a range of concentrations between 0.02-7.17 ng/g. No significant differences were observed in placental PFAS levels based on infant sex. We observed a negative association between placental PFOS concentration and birth weight, but only in male infants. This finding is similar to previously reported studies that examined associations between maternal serum PFAS and birth weight.

PL 2435 Replacement Per- and Polyfluoroalkyl Substances (PFASs) Are Potent Modulators of Lipogenic and Drug Metabolizing Gene Expression Signatures in Primary Human Hepatocytes

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Per- and polyfluoroalkyl substances (PFAS) are a family of toxicants that are used in fire-fighting foams and as well as many heat-, stain- and water-resistant products. With about 4000 PFASs estimated to be in use in the commercial market, there is a serious lack of knowledge regarding the toxicodynamic effects and adverse health outcomes of replacement PFAS in humans. A subset of PFASs, called perfluoroalkyl acids (PFAA), are fully-fluorinated sulfonic and carboxylic acids, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). PFAAs have been shown to induce liver steatosis in rodents and monkeys. However, it is unclear whether PFOS or PFOA exposure causes steatosis in humans, with some existing evidence that PFOS and PFOA treatment can induce expression of lipid metabolism and synthesis genes in human hepatocytes. We hypothesized that PFOS and PFOA, along with other prevalent PFASs, could induce gene expression of lipid metabolism and synthesis along with lipid deposition in cryopreserved human hepatocytes. Cryostax 5-donor pool of cryopreserved human hepatocytes were plated onto 96-well plates. 24 hrs after plating, cells were treated with various PFAAs and replacement PFASs at various concentrations (0.25, 2.5, and 25 µM) or 0.1% DMSO vehicle in media. After 48 hrs of treatment, cell lysates were

collected and the relative abundance of 35 target transcripts and 4 housekeeping genes was measured using a custom QuantiGene 2.0 plex assay. A second set of hepatocytes were stained with DAPI and Nile Red and fluorescence was quantified. Treatment with different PFAAs (3 sulfonates and 12 carboxylates) and alternative PFASs for 48 hrs modulated gene expression for 6 nuclear factors related to drug metabolism and lipid regulation, as well as induction in 20 drug metabolism, lipid metabolism, cholesterol metabolism, lipid synthesis, and lipid transport genes. Shorter chain PFAAs (C4 sulfonate and C4-C7 carboxylates) and PFAS replacements induced significant liver lipid accumulation and had gene activation at much lower concentration than legacy PFAAs. Hepatic lipid accumulation was not observed in treatments with longer chain PFAAs (C6-C8 sulfonates and C6-C12 carboxylates, except C10, C13, and C14). Overall, PFAS were highly transcriptionally active. Although alternative PFASs have shorter half-lives, shorter chain PFAAs and replacement PFAS were much more potent in our assays, and this work suggests health effects of these compounds need to be more critically evaluated. We hope to correlate the observed trends in chain length to a biological processes that may help identify priority PFASs.

2436 Investigating the Toxic Effects of Perfluoroctanoates PFOA, PFOS, and PFHx on Hepatic Toxicity and Cytochrome P450 Metabolism

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Per- and polyfluoroalkyl substances (PFASs) are a group of chemicals which have been extensively manufactured and used in a variety of consumer and industrial products. Because of their chemical properties, PFAS persist in the environment and bioaccumulate in the human body. Exposure to PFAS are associated with many adverse human health effects, including reproductive, developmental, liver, kidney and immunological effects in people as well as laboratory animals. While these chemicals are still widely used and persist in the environment, the mechanisms behind PFAS toxicity is not well understood. Here, we utilized three species of PFAS, including perfluorooctanoic acid (PFAS), perfluorooctanesulfonic acid (PFOS) and perfluorohexane-1-sulphonic acid (PFHx) to investigate specific toxicity to human hepatocytes *in vitro*. We find that exposures that range up to 250mM cause significant toxicity to HepG2 hepatocytes, as measured by LDH release assay. Moreover, we evaluated CYP3A7 and CYP19A1 (aromatase) activites following PFOS and PFAS exposure. Based on the perfluorooctanoates resemblance to endogenous lipid substrates, and their known inhibition of hepatic drug metabolizing CYP enzymes, it was our hypothesis that PFOA and PFOS would effectively inhibit the metabolism of CYP3A7 and CYP19A1. We found that both PFOA and PFOS inhibited the metabolism of the fluorescent substrate dibenzylfluorescein (DBF) by CYP3A7 and CYP19A1. The IC_{50} values for CYP3A7 inhibition were 21 μM for PFOS and 75 μM for PFOA. For CYP19A1 the IC_{50} value was 41 μM for PFOS. Interestingly, both PFOS and PFOA bound to CYP3A7 in a reverse Type I manner, suggesting possible displacement of the axial water ligand and ligation to the heme iron. Inhibition of the enzymatic activity of these two enzymes points to a mechanism of action in the liver. Overall, these data suggest that the direct effects of PFAS exposure may affect not only effect liver toxicity, but the overall ability of the tissue to perform essential metabolic function.

2437 Perfluorooctanoic Acid Induces Liver and Serum Dyslipidemia in a Humanized PPARα Mouse Model Fed an American Diet

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Americans are exposed daily to mixtures of perfluoroalkylated substances (PFAS) in drinking water, food, air, dust in their homes, and direct use of consumer products. Increased concentrations of serum total cholesterol and low density lipoprotein cholesterol (LDL-C) are among the best supported endpoints by epidemiology studies. Dyslipidemia (elevated triglycerides, decreased high density lipoprotein cholesterol) and increased serum LDL-C are major contributors to cardiovascular disease, the leading cause of mortality in the US. Despite evidence that human PFAS exposure is associated with dyslipidemia, these effects of PFAS have received little toxicological research focus, largely because there is no suitable animal model. Mechanisms by which PFAS cause lipid-disrupting metabolic effects are not well understood, but evidence for interaction with human peroxisome proliferator activated receptor α (PPARα), an essential mediator of cholesterol and lipid homeostasis is one logical molecular initiating event. Polymorphisms in human PPARα are associated with changes in serum cholesterol. Our data show that PFAS activate human PPARα transcriptional activity. Mice expressing human PPARα and fed a diet based on the "What we eat in America" analysis provide a new animal model needed for PFAS-induced dyslipidemia research. Perfluorooctanoic acid (PFOA) exposure in drinking water (3.5 mg/l) for 6 weeks resulted in a serum PFOA concentration of 48 ± 9 μg/ml, a serum level approximately 2-fold higher than that found in fluorochemical workers. PFOA exposure increased liver mass, increased lipid content, perturbed the liver and serum lipidomes and induced PPARα target gene expression with sex-dependent effects. Effects were significantly altered in PPARα knockout mice. A literature-based analysis of effects of PFOA on serum triglyceride and cholesterol across multiple mouse models revealed a non-monotonic dose response. The humanized PPARα mouse model fed an American diet is an important new model for examining PFAS-induced dyslipidemia.

2438 Ecological Toxicity Mapping of Per- and Polyfluoroalkyl Substances (PFAS) with Ecotoxicology Knowledgebase Protocols

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Given the persistence and wide distribution of some per- and polyfluoroalkyl substances (PFAS) in the environment, there is a recognized need to characterize potential ecological impacts and risks of these substances. The goal of this work was to identify and describe available empirical evidence for ecological effects of PFAS using the systematic, well-established search and curation protocols of the ECOTOXicology Knowledgebase (ECOTOX, www.epa.gov/ecotox), and then identify potential toxicity pathways for this group of compounds. ECOTOX is a publicly available web-based database that has curated ecologically-relevant toxicity data for over 30 years to support chemical decision making. Comprehensive literature searches with over 300 chemical names and CASRNs were conducted in 2018-2019 to update PFAS data in ECOTOX. From the resulting 15,000 references, title and abstract screening identified over 500 papers that met inclusion criteria for ECOTOX (e.g., ecologically-relevant species, verifiable CASRN, endpoint and control reported). Full-text review has identified 250 acceptable papers to-date and is on-going. All pertinent study details were extracted from each acceptable paper [species, chemical(s), test methods, and toxicity results]. ECOTOX now contains fully curated ecotoxicological data for 96 PFAS from 437 references with 264 unique biological test species. Most data in these studies were for PFOS and PFOA in a handful of species (e.g., zebrafish, earthworm, chicken), with 30% traditional growth/reproduction/mortality toxicity endpoints and nearly 40% biochemical or genetic effects reported. With genotypic data for the common test species, *Danio rerio*, ontologies (e.g., Gene Ontology) and pathways databases (e.g., REACTOME, KEGG) were used to identify multiple candidate pathways potentially implicated in PFAS toxicity, including lipid metabolism, carbohydrate metabolism, and endocrine system. Where genotypes are not available, the toxicity responses will be mapped to pathways using ontology-based semantic analysis. The ecological toxicities for PFAS identified and curated through these efforts can inform ecological risk assessments, development of Adverse Outcome Pathways, species extrapolation, and other applications. *The contents of this presentation do not reflect US EPA policy.*

2439 Effects of Developmental Exposure to Perfluoroalkyl Substances on Brain and Heart Innervation in Northern Leopard Frogs

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Perfluoroalkyl substances (PFAS), such as perfluorooctane sulfonate (PFOS), are compounds that have been used in a variety of products like aqueous firefighting foams, stain and water repellents, nonstick cookware, carpets, and clothing. Discharge from manufacturers and usage has led to PFAS presence in drinking water and >99% of human serum, where half-lives are in the order of years. Research has shown PFAS accumulation in the brains of wildlife such as polar bears and North Atlantic pilot whales and that 10-100x more PFOS accumulates in the brain compared to other tested PFAS. However, research on the effects of exposure to these compounds on the brain, especially during development, is lacking. Prior work from our group showed that exposure to 100-1000 ppb PFOS selectively altered dopamine neurotransmission. We hypothesized that environmentally-relevant mixture doses would produce similar toxicity and decrease sympathetic innervation of the heart. To determine effects, we aqueously exposed Gosner stage (GS) 25 Northern Leopard frogs (n=25/tank, 4 tanks/treatment) to 10 ppb PFOS or a PFAS mixture (4 ppb PFOS, 3 ppb perfluorohexane sulfonic acid, 1.25 ppb perfluorooctanoic acid, 1.25 ppb perfluorohexanoic acid, and 0.5 ppb perfluoro-n-pentanoic acid).

Nine tadpoles per tank were sacrificed after 30 days of exposure for brain neurochemical analysis. Remaining tadpoles were collected at GS 42 and allowed to grow to GS 46 before the brain and heart were collected. Weight, length, GS, and time to development were recorded for each frog, PFAS were quantified using mass spectrometry, and neurotransmitters were quantified using High Performance Liquid Chromatography and with the Invitrogen Amplex Red Acetylcholine Assay. Preliminary data suggests that after 30 days, a significant decrease was evident in PFAS-treated frogs, whereas no significant differences in physiological data or other neurotransmitter levels were detectable. Trends toward decreased acetylcholine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid (HVA) suggest further analyses. PFOS-exposed frogs were longer than controls at metamorphosis, although time to metamorphosis did not significantly differ among groups. There were no significant differences in neurotransmitters in the brain or heart, although there were trends to decreased dopamine and increased dopamine turnover in PFOS-treated brains and increased HVA and norepinephrine in hearts. Overall, preliminary data suggests that PFAS could be affecting growth and causing modest changes in neurotransmitter levels, which could lead to detrimental effects if exposed to another neurotoxic chemical.

PL 2440 The Effects of E-cigarette and Cigarette Use on the Nasal Microbiome

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Electronic cigarettes (e-cigarettes) are gaining popularity as an alternative to smoking traditional cigarettes. E-cigarettes are often perceived as safer than cigarettes, but the effects of e-cigarette use on the respiratory system have not been fully characterized. One area of research that has not been explored in the context of e-cigarette use is the potential effect of e-cigarettes on the respiratory microbiome. The respiratory microbiome plays a key role in respiratory host defense, and alterations in the respiratory microbiome (dysbiosis) have been associated with diseases such as asthma, chronic obstructive pulmonary disease, chronic rhinosinusitis, and cystic fibrosis, as well as with smoking cigarettes. To determine whether e-cigarette use causes respiratory microbiome dysbiosis, we sampled the nasal microbiomes of adult e-cigarette users, smokers, and non-smokers using a non-invasive absorptive strip to collect epithelial lining fluid. We then used high-throughput sequencing of the bacterial 16S rRNA gene from the strips and microbiome analysis pipelines to identify bacteria present and analyze the bacterial composition of the nasal microbiome. Our results indicate that alpha diversity (within individual) is significantly greater in male subjects than in female subjects and that this relationship is particularly pronounced in the e-cigarette user group. We also found that beta diversity measures (between individuals) clustered significantly by sex and exposure group. We observed a significant increase in bacteria from genera *Staphylococcus, Streptococcus, Corynebacterium,* and *Propionibacterium* in cigarette users, which is in agreement with previous studies. In the e-cigarette group, there was a significant decrease in relative abundance of bacteria in the genera *Propionibacterium, Moraxella,* and *Shuttleworthia*. Our results indicate that e-cigarette use can alter the nasal microbiome and that this alteration is different from the microbiome dysbiosis observed in association with cigarette use. Overall, these data provide insight on the effects of sex as well as e-cigarette and cigarette use on the nasal microbiome, which contributes to our understanding of the respiratory effects of e-cigarette use.

PL 2441 Electronic Cigarette Aerosol-Induced, Oxidative Stress-Mediated Lung Epithelial Cell Injury Is Alleviated by N-Acetyl Cysteine

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Emerging evidence indicates that exposure to electronic-cigarette (e-cig) aerosols induces vaping-associated pulmonary illness (VAPI), potentially leading to death. The cellular and molecular mechanisms underlying these effects, however, are unknown. We previously showed *via* an air-liquid interface (ALI) exposure model that exposure of human bronchial epithelial cells (H292) to butter-flavored e-cig aerosol increased reactive oxygen species (ROS) generation and up-regulated expression of *HMOX1*. N-acetyl cysteine (NAC) is an antioxidant that protects against ROS-mediated lung injury. In the present study we asked whether pre-treatment with NAC can alleviate e-cig aerosol-induced oxidative stress-mediated lung epithelial cell injury. H292 cells were pre-treated with or without 5mM NAC for 12 hours before a single 2-hour exposure to each of two different flavored-e-cig aerosols (butter and cinnamon). Cells were exposed to the e-cig aerosols at the ALI *via* a Vitrocell exposure system connected to a third-generation e-cig device. Cytotoxicity, epithelial cell barrier integrity (TEER), gene expression, and markers of oxidative stress and DNA damage were assessed. Exposures to butter- and cinnamon-flavored e-cig aerosols alone significantly reduced the numbers of viable cells and reduced TEER; cinnamon e-cig aerosols also increased levels of ROS and lactate dehydrogenase (LDH). NAC pre-treatment reduced all these responses to control levels. Further, cinnamon-flavored e-cig aerosols upregulated expression of α7*nAChR, CYP1A1*, *CYP1B1*, *ALDH3A1, NOX1, NOX2, DUOX2, CCL2,* and *TNF-α,* and downregulated expression of *NF-kB1, STAT6,* and *NRF2*. Dysregulation of all these genes was prevented by NAC pre-treatment. Only cinnamon-flavored e-cig aerosols augmented the cell media levels of heme oxygenase-1 (HO-1), a marker of oxidative stress, and 8-hydrodeoxyguanosine (8-OHDG), a marker of DNA damage. Those increases were prevented by NAC pre-treatment. Pre-treatment with NAC alleviate the oxidative stress-mediated damage to lung cells induced by e-cig aerosols. These data suggest that oxidative stress is critical to the mechanism of e-cig aerosol toxicity.

PL 2442 Chemical and Toxicological Characterization of Vaping Products from Different Viscosity Enhancers in E-liquids

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There have been an increasing number of reports on the detrimental vaping-related incidents, such as lung injury, lipid pneumonia, and the occurrence of seizures, which illicit a great health concern regarding the usage of vaping products including e-cigarettes and cannabis. The prevalence of vaping among adolescents has increased dramatically within the past decade for the reduced consumption of nicotine and lack of combustion needed to consume the product during vaping. The major components of vape juices and e-liquids are the viscosity enhancers, the percentage of which can reach up to 100%. Previous studies have reported that the thermal degradation and oxidation of commonly used viscosity enhancers (e.g., propylene glycol) can produce a large amount of highly toxic aldehydes (e.g., formaldehyde and acetaldehyde). Recently, a variety of new viscosity enhancers have been introduced into the market. However, to date, the chemical compositions and toxicological characterization of vaping products from these viscosity enhancers are largely unknown. This pilot study aims to provide a molecular and toxicological characterization of e-cigarette vaping products from eight commonly used viscosity enhancers, including propylene glycol, glycerin, medium-chain triglyceride (MCT) oil, triethyl citrate, squalane, vitamin E (tocopherol), vitamin E acetate (tocopheryl acetate) and a popular terpene-based diluent. The vaping products in both gas and particle phase were collected into two tandem impingers. Samples collected in isopropyl alcohol and o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) aqueous solution were applied to GC-MS for chemical analysis. Samples collected in LHC-9 cell culture media were applied for cell proliferation and cytotoxicity evaluation in human epithelial lung cells (BEAS-2B). Our study has shown that these viscosity enhancers can decompose and be oxidized during vaping. Furthermore, the cytotoxicity of the vaping products from these viscosity enhancers will be linked to the corresponding chemical compositions. The results of this study will inform the general public of potential vaping-related health risks.

PL 2443 Chronic Use of Electronic and Tobacco Cigarettes Alters Oxidized Lipids in Healthy Young Adults

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Sponsor: O. Hankinson

Electronic cigarettes (EC) have gained unprecedented popularity in recent years especially among the youth with over 3.6 million users in the US. Recent published case reports have detailed range of severe pulmonary illnesses associated with increased oxidative stress and/or inflammation among EC users. Here, we aimed to determine whether chronic EC use leads to changes in antioxidant defense and/or lipid peroxidation in plasma as compared to chronic tobacco cigarette (TC) smokers. We enrolled EC users (n=32), TC smokers (n=29) and control non-smokers (n=45), with mean ages of 28, 27.4 and 27.5 years, respectively. Plasma levels of oxidized fatty acid metabolites were assessed by liquid chromatography-mass spectrometry. Paraoxonase-1 (PON-1) enzymatic activity and total antioxidant capacity (TAC) were also evaluated in the plasma. Plasma levels of arachidonic acid (AA) and linoleic acid (LA) were significantly higher in TC smokers as compared with EC users ($p<0.01$). On the other hand, the levels of oxidized LA metabolites (9-hydroxyoctadecadienoic acid (9-HODE) + 13-HODE) in EC users (5.05 ± 0.41 ng/ml) and TC smokers (4.43 ± 0.43 ng/ml) were markedly lower ($p<0.01$) as compared to controls (9.85 ± 1.06 ng/ml). Surprisingly, a significant increase ($p<0.001$)

was observed in plasma total antioxidant capacity in TC smokers (1.62 ± 0.07 mM Trolox) when compared to controls (1.37 ± 0.03 mM Trolox) with a similar trend in EC users (1.47 ± 0.03 mM Trolox) when compared to controls (p=0.14), with no differences in either PON-1 or arylesterase activities. In conclusion, chronic use of either EC or TC smoking over a year leads to reduced plasma levels of oxidized fatty acid metabolites (9-HODEs and 13-HODEs) in healthy young adults, likely due to homeostatic compensatory anti-oxidant responses triggered by EC and TC pro-oxidant effects.

PL 2444 Prenatal E-cig Aerosol Exposure Leads to Sex-Dependent Abnormal Extracellular Matrix (ECM) Remodeling and Myogenesis in Offspring Mice

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E-cig vaping is a serious concern, especially since many pregnant woman who smoke consider it safe. However, little is known about health risks of prenatal exposure to unflavored e-cig aerosols later in life. The goal of this toxicological study is to determine the effects of prenatal exposure of mice to e-cig aerosols on lung development, particularly on ECM remodeling and myogenesis. Pregnant CD-1 mice were exposed to unflavored e-cig aerosols, with or without nicotine; at 6-wk-of-age, male and female offspring were examined. Lung tissue was histologically-analyzed and total RNA and protein were isolated for quantification of lipogenesis/myogenesis and ECM remodeling biomarkers. Compared with sex- and age-matched air control offspring, female mice exposed prenatally to e-cig aerosols (with or without nicotine) demonstrated increased protein levels of the myogenic proteins: fibronectin, LEF-1, and E-cadherin, whereas males exposed prenatally to e-cig aerosols with nicotine had decreased E-cadherin, but increased PPARγ protein levels. Pertinent lipogenesis/myogenesis lung mRNAs (*ADRP*, *CNN1* and *ACTA2*) were also dysregulated in a sex-dependent manner. Interestingly, ECM regulator PAI-1 was significantly increased in females exposed prenatally to e-cig aerosols with nicotine, whereas it was up-regulated in males following exposure to e-cig aerosols with or without nicotine. MMP9, a downstream target of PAI-1, was down-regulated in both sexes, while MMP2 and TIMP1 were increased only in females exposed to e-cig aerosols with or without nicotine. No differences in lung histology were observed between treatment groups. Our data show that mice exposed prenatally to unflavored e-cig aerosols could be predisposed to developing pulmonary disease later in life in a sex-dependent, but nicotine-independent manner. These findings suggest that e-cig use during pregnancy is not safe and could increase the offspring's propensity for interstitial lung diseases later in life. *NIH 1R01HL135613; NIH HL127237; TRDRP 27IP-005; NIH P30 ES000260.*

PL 2445 Short-Term JUUL and Third-Generation E-cig Aerosol Exposure Dysregulates Biotransformation and Inflammation-Related Genes in Murine Macrophages Exposed at the Air-Liquid Interface

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Electronic nicotine delivery systems (ENDS), including electronic-cigarettes (e-cigs) and JUUL-like devices, are now considered the most commonly used form of tobacco products among youth in the United States. The prevalence of ENDS usage is currently 2.1 million among middle and high school students, fueling concerns over the adverse health effects related to ENDS use. Emerging evidence suggests that ENDS aerosols affect local immune function of key protective cells, including alveolar macrophages, the lungs' first line of defense against inhaled xenobiotics. The aim of the present study was to evaluate the *in vitro* toxicity and gene expression of two popular JUUL and e-cig flavors (crème brûlée and mango) using an *in vitro* physiologically-relevant exposure model. JUUL and e-cig aerosols were generated using a peristaltic pump or a third-generation e-cig device. Applying standard vaping topography profiles, murine macrophages (RAW 264.7) were exposed to JUUL or e-cig aerosols at the air-liquid interface (ALI) for 60 minutes. Filtered-air was used as a control. Cell viability, cytotoxicity and membrane damage, nitric oxide (NO) production, and gene expression were assessed. Although crème brûlée and mango-flavored JUUL and e-cig aerosol exposures did not affect cell viability or LDH activity, JUUL and e-cig aerosols in both flavors resulted in ≥ 50% reduction in NO production compared to air controls. This was supported by the downregulation of *iNos*, a key gene that plays a role in macrophage inflammatory responses. Moreover, crème brûlée-flavored aerosols from both devices upregulated the expression of α_7*nAChR, Cyp1a1*, *AhR*, *Tgf-β* and *Mmp12* genes, while down-regulating *Ifn-γ* and *Il-6*. Exposure to mango-flavored aerosols from both devices upregulated the expression of α_7*nAChR,* while down-regulating *Il-6 and Cyp1b1*. Only e-cig mango exposures resulted in up-regulation of *Cyp1a1* and down-regulation of *AhR*, while JUUL only downregulated *Tgf-β*. These results suggest that flavored e-liquids, when aerosolized through e-cigs or JUUL devices, may adversely affect immune and respiratory health through alteration of key inflammatory molecules and associated genes. Moreover, all flavors may not be equal in toxicity, as flavors composed of a mixture of constituents, like crème brûlée, may be more detrimental to macrophages than other flavors, e.g. mango. Our future studies intend to investigate the underlying mechanisms associated with these effects using *in vitro* and *in vivo* models.

PL 2446 Impact of E-cigarette Flavoring Agents on Activity of CYP2A6, the Primary Nicotine-Metabolizing Enzyme

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Flavorings in e-cigarette liquids (e-liquids) are a primary driver of e-cigarette use among teens. While recent studies have investigated pulmonary toxicities associated with exposure to flavoring agents in e-cigarettes, little research has been conducted on the impact of flavoring agents on the pharmacokinetics of nicotine, the primary addictive compound of e-cigarettes. Utilizing recombinant microsomal CYP2A6, the enzyme primarily responsible for the metabolism of nicotine, we screened 3 e-liquids - Strawberry Poptart (SP), Apple Watermelon (AW), and Flamethrower (FT) - at concentrations ranging from 0.000031% to 0.125% (v/v), for CYP2A6 activity inhibition. CYP2A6 activity was determined by measuring the formation of the fluorescent metabolite of 3-cyano-7-hydroxycoumarin, a substrate of CYP2A6. Incubation with FT and SP strongly inhibited CYP2A6 at concentrations as low as 0.00038% and 0.0058%, respectively. AW exhibited negligible CYP2A6 inhibition. Mass spectrometry was conducted to identify concentrations of flavoring agents in flavored e-cigarette liquids that inhibited CYP2A6. Recombinant microsomal CYP2A6 was subsequently exposed to flavoring agents at concentrations ranging from 0.03 μM to 500 μM. Of the flavoring agents tested, cinnamaldehyde and benzaldehyde were found to be the most potent inhibitors of microsomal CYP2A6, with identified IC_{50} values of 1.1 μM and 3.0 μM, respectively. To confirm our findings in a cell-based system, we transduced BEAS-2B cells to overexpress CYP2A6 and subsequently exposed them to e-liquids and flavoring agents in the presence of coumarin, a CYP2A6 probe substrate. Exposure to SP, FT, and aromatic aldehydes utilized as flavoring agents significantly reduced the metabolism of coumarin by CYP2A6 at concentrations consistent with what was observed using recombinant microsomal CYP2A6. In summary, these data indicate that some flavoring agents in e-cigarettes have the capacity to inhibit CYP2A6 activity, the enzyme responsible for catabolizing nicotine. This may increase serum concentrations of nicotine and have implications for the risks associated with e-cigarette use. *This abstract may not reflect official US EPA policy.*

PL 2447 E-cigarette Vapor Decreases Bone Marrow Hematopoietic Stem and Progenitor Cells

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Environmental exposure to fine particulate matter (PM) is associated with increased circulating endothelial progenitor cells. Electronic nicotine delivery system (ENDS) or E-cigarette use has increased exponentially in recent years and while a few studies indicate inflammatory and pro-oxidant effects of vaping in the respiratory system, there is limited evidence of vaping effects on the hematopoietic system. Since e-cigarette usage generates fine and ultra-fine PM, we hypothesize that chronic e-cigarette smoke exposure can alter hematopoietic stem and progenitor cell (HSPC) frequency and function via chronic inflammation. Female C57BL/6J mice were exposed via nose-only inhalation to ENDS-vapor containing nicotine for 2 months. At euthanasia, bone marrow was collected and cells stained with HSPC-specific antibodies for flow cytometry. Data was analyzed using FlowJo software. After 2 months of ENDS-exposure, we observed a decrease in hematopoietic stem and progenitor cells (HSPCs). Specifically, the lineage negative, c-Kit positive and Sca-1 positive (LKS) population was significantly reduced in the ENDS-exposed group with concomitant reduction in common myeloid progenitor (CMP) and granulocyte-monocyte progenitor (GMP) populations. There was also a

slight decrease in the frequency (p=0.065) and absolute (p=0.051) numbers of long-term HSCs. Interestingly, this reduction did not lead to changes in peripheral blood cell counts nor spleen weight. We also determined the effect of systemic LPS administration post-exposure on the proliferation of HSCs and observed no significant differences between ENDS and air exposed groups. Our study provides important evidence of changes in bone marrow HSPC populations following chronic E-cigarette exposure that can eventually lead to myelosuppression. Incidentally, combustible cigarette smoke exposure has also been shown to decrease HSPC numbers and it is striking that E-cigarette vapor exerts similar effects, although it is possible that these effects are nicotine-dependent. Future work includes determining HSC reconstitution in mice transplanted with bone marrow from the ENDS-exposure group.

PL 2448 Acute Exposure to E-cigarette Aerosols Alters Cardiac Conduction and Autonomic Balance and Induces Arrhythmia in Mice

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E-cigarette (e-cig) use has rapidly increased, especially among youth and amidst assumptions that e-cigs are safer than smoking. Vaping has been linked to adverse cardiopulmonary effects, but the full extent of effects remains unknown. Several constituents in e-cigs may increase the risk for adverse cardiopulmonary outcomes partly by disturbing cardiac electrophysiology and autonomic nervous system (ANS) regulation. E-cig aerosols will differentially induce pro-arrhythmic changes in cardiac conduction and autonomic balance in mice depending on the presence of nicotine and flavors. Electrocardiograms (ECGs) were collected by telemetry in 5 healthy male mice (C57BL/6) exposed for 6 hours to clean air or various e-cig aerosols (9-minute puff sessions every 18 minutes) from JUUL e-liquids (Virginia [VA] Tobacco, Mango, or Menthol at ≈5% nicotine benzoate), or a nicotine-free mixture of propylene glycol and vegetable glycerin solvents (PG:VG, 30:70 ratio). ECG morphology, heart rate variability (HRV), and arrhythmias were analyzed by mixed models with $P < 0.05$ (vs. Air) for all reported effects. All e-cig aerosols increased ventricular arrhythmias throughout exposure, with PG:VG inducing the greatest effects. PG:VG also markedly increased advanced supraventricular block arrhythmias and uniquely decreased heart rate. In contrast, aerosols from nicotine-containing flavored e-liquids increased heart rate and decreased HRV, suggesting increased sympathetic modulation. E-cig aerosols from PG:VG and VA Tobacco prolonged QTc interval, suggesting arrhythmogenic impairments in repolarization regardless of nicotine. Mango and Menthol accelerated atrial (P duration) and atrioventricular (PR interval) conduction, whereas VA Tobacco accelerated atrial conduction without commensurate atrioventricular changes. E-cigs may increase risk for cardiac arrhythmia through e-liquid solvents, which when thermally aerosolized generate toxic aldehydes and particulates. Nicotine and flavor chemicals may modify the cardiac and autonomic impacts of e-cigs. Further studies are needed to determine how e-cig aerosols induce cardiac arrhythmia and whether these effects translate to cardiac morbidity and mortality in humans.

S 2450 Immunosuppressive and Anti-inflammatory Activity of Cannabis and Cannabinoids: Adverse or Therapeutic?

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Cannabis is currently the most widely used illicit drug in the US, with an estimated 35 million regular users, or people using cannabis more than twice a month. In addition, medicinal cannabis and cannabinoid-based therapeutics have been gaining prominence, with 33 states, four (out of five) permanently inhabited US territories, and the District of Columbia having legalized medical marijuana as of January 2019. Cannabinoids are a family of more than 60 structurally related compounds initially identified in *Cannabis sativa*. Certain cannabinoids, including the primary psychotropic congener Δ^9-tetrachydrocannabinol, are widely established as possessing immune modulating activity. Over four decades of investigation have revealed that virtually every immune cell type responds to modulation by cannabinoids. The biological activity of cannabinoids is mediated primarily, but not exclusively, through two G-protein coupled receptors termed *cannabinoid receptors* (CB1 and CB2). CB1 is expressed primarily in the CNS but can be found in most peripheral tissues, including immunocompetent cells. CB2 is found primarily in the periphery, with the exception of microglial cells, and is expressed most notably by immune cells. The identification and cloning of CB1 and CB2 led to the discovery of endogenous cannabinoids and the endocannabinoid system, which appears to influence a diversity of physiological processes, including immune responses. The widespread recreational use of cannabis (legal in 11 states and DC) has raised concerns for adverse effects. By contrast, cannabinoid receptors, and in particular CB2, represent potential therapeutic targets. The goal of this session is to discuss both the potential adverse as well as the therapeutic aspects associated with cannabinoid-mediated immune modulation in specific contextual settings based on experiments performed in animal and human model systems. The Symposium will begin with an introduction to cannabis and cannabinoids, followed by presentations that address the role of cannabinoid receptors in NK cell and innate lymphoid type 2 (ILC2) cell responses to an airway allergen; the effect of cannabinoids on the gut microbiome and downstream immunological consequences; the ability of cannabidiol, a nonpsychotropic cannabinoid that was recently US Food and Drug Administration approved for epilepsy, to inhibit immune responses in the periphery to attenuate autoimmune disease; and cannabinoid-mediated suppression of immune responses implicated in HIV-associated neuroinflammation. After this session, attendees will have a better understanding of the underlying biology of immune modulation by cannabinoids with respect to both adverse as well as potential therapeutic applications.

S 2451 History of Cannabis Use and Introduction to Cannabinoids

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Cannabis is, and will remain, in the news for the foreseeable future. The term cannabis refers mainly to the marijuana plant, *Cannabis sativa*. Cannabinoids are structurally similar plant-derived chemicals in cannabis that possess similar actions to endogenous cannabinoids, termed *endocannabinoids*. Plant-derived cannabinoids and endocannabinoids exhibit activity, in part, via cannabinoid receptors, CB1 and CB2. While expression of both receptors is ubiquitous, CB1 is highly expressed in the central nervous system and CB2 is highly expressed in immune cells. The two best-characterized plant cannabinoids are Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is the major psychotropic compound in marijuana, while CBD does not produce a high, which is likely due to the fact that THC exhibits affinity for the CB1 receptor while CBD does not. A similar pattern has emerged for CB2: THC possesses affinity for CB2 and some of the immune effects of THC are mediated by CB2, while CBD does not exhibit high affinity for CB2. In fact, identification of the receptor(s) mediating the immune effects of CBD remains elusive. Despite their distinct pharmacology, THC and CBD exhibit anti-inflammatory and immune suppressive effects. It also is clear that endocannabinoids must possess immune modulatory activity, as CB1 and/or CB2 regulate immune function. Whether the immune suppression by cannabinoids should be considered adverse or therapeutic will be focus of this Symposium.

S 2452 CB2 Receptors Impact the Development of Allergic Airway Inflammation

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In allergic asthma, inhalation of environmental allergens such as the house dust mite (HDM) effectively activates both innate and adaptive immunity in the lung. Endogenously generated cannabinoids acting via CB2 receptors play important roles in both homeostatic and inflammatory processes. Our studies revealed that CB2-deficient (CB2-/-) mice had increased numbers of pulmonary natural killer cells (NK cells) that were inversely associated with the number of group 2 innate lymphoid cells (ILC2s) compared with C57BL/6 wild-type animals. Remarkably, CB2-/- mice were markedly less responsive to HDM allergen inhalation than wild-type counterparts since the knockout mice failed to develop many of the cardinal features of asthma. Notably, this included diminished levels of airway hyperreactivity, mucus production, eosinophilic inflammation, and CD4+ lymphocyte infiltration, as well as depressed levels of type 2 cytokines. This phenomenon was corroborated when wild-type mice were treated with a CB2-specific antagonist that caused a pronounced inhibition of HDM-induced airway inflammation and mucus secretion. NK cells were responsible for the attenuated inflammatory responses because depletion of NK cells *in vivo* in CB2-/- mice restored both the HDM-induced allergic inflammation and ILC2 numbers in the lungs. Collectively, these data demonstrate a role for CB2 receptors in the development of allergic airway inflammatory responses by regulating the number and properties of NK cells resident in the lungs.

S 2453 Cannabinoid-Induced Changes in Gut Microbiota and Suppression of Inflammation

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Exposure to Staphylococcal enterotoxin B (SEB) triggers cytokine storm, acute inflammatory lung injury (ALI), food poisoning, toxic shock syndrome, and often death. Because cannabinoids act as potent anti-inflammatory agents,

we tested the effect of Δ9-tetrahydrocannabinol (THC) on SEB-mediated ALI. Additionally, we tested if the anti-inflammatory effects of THC result from alterations in the microbiota in the gut and in the lungs. SEB administration in mice caused ALI and 100% mortality, while all THC-treated mice survived following suppression of inflammation in the lungs by inducing Tregs and myeloid-derived suppressor cells. We noted significant microbial dysbiosis following SEB treatment in the lungs, specifically with an increase in abundance of various taxa belonging to Proteobacteria, whereas THC-treated SEB-exposed mice showed a reversal in this class. Furthermore, the SEB+THC treated mice also exhibited an increase in the butyrate-producing *Ruminococcus gnavus*. Interestingly, similar changes in *Ruminococcus gnavus* also were observed in the colon in SEB+THC treated group, thereby suggesting a critical role for gut-lung axis in health and disease. We also found that the beneficial butyrate levels were significantly higher in the colonic flush of THC-treated mice that were exposed to SEB when compared with controls. In addition to THC, we found that endocannabinoids were also able to protect mice from SEB-mediated ALI, which also was associated with changes in microbiota. Together, our studies demonstrate that cannabinoids attenuate cytokine storm induced by super antigens such as SEB, through regulation of microbiota and their metabolites, which suppress inflammation through gut-lung axis.

S 2454 Cannabidiol Suppresses IFNγ Production and Neuroinflammation in the EAE Model

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Cannabidiol (CBD) is a plant-derived cannabinoid similar in structure to the psychoactive cannabinoid, Δ9-tetrahydrocannabinol (THC), although CBD does not produce a high. Despite their differences in psychotropic effects, both THC and CBD are immune suppressive. We utilized the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis to determine effects and mechanisms by which CBD suppresses immune function. EAE disease was initiated on day 0 followed by five days of dosing with CBD by oral gavage. CBD significantly reduced disease severity at day 18. T cell responses in the spleen, lymph nodes, spinal cord, and cerebellum were assessed at day 3, 10, and 18 following disease initiation. Although it has been shown that CBD can induce T regulatory cells (Tregs) *in vitro*, we did not detect a significant increase in Tregs any time, at least in spleen and lymph nodes. However, IFN-γ production was significantly inhibited by CBD in the spleen at day 10, which preceded robust suppression of inflammation in the spinal cord and cerebellum at day 18. Together, these data demonstrate that CBD is effective in attenuating autoimmune disease and suggests that inhibition of immune responses in the periphery early might contribute to later attenuation of inflammation in the central nervous system as part of the mechanism for disease attenuation.

S 2455 Cannabinoids Suppress Immune Responses Implicated in HIV-Associated Neuroinflammation

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An estimated 37 million people worldwide are infected with human immunodeficiency virus (HIV). Combined antiretroviral therapy has turned HIV into a chronic infection with significantly longer life expectancy. New health issues have surfaced as HIV patients live longer. Specifically, 50% of HIV-infected (HIV+) individuals exhibit neurocognitive impairment, termed *HIV-associated neurocognitive disorders* (HAND). A key event leading to HAND is persistent low-level chronic neuroinflammation resulting in neuronal damage. A major contributor to HIV-induced neuroinflammation is activated monocytes, which are significantly elevated in patients with HIV-associated dementia. Activated, $CD16^+$ monocytes are infected by HIV in the periphery and migrate across the blood-brain barrier (BBB) to release HIV virions and neurotoxic and proinflammatory factors. Before entering the CNS, resting ($CD16^-$) monocytes transition into $CD16^+$ through poorly understood mechanisms including elevated interferon alpha (IFNα). Interestingly, cannabis, which has constituents (e.g., Δ^9-tetrahydrocannabinol (THC)) possessing immune suppressive and anti-inflammatory activity, is widely used (approximately 25%-37%) by HIV patients. Beneficial versus deleterious effects of cannabinoid therapy in HIV patients remains unknown. Our results show that THC suppresses IFNα-mediated $CD16^-$ to $CD16^+$ monocyte transition as well as IL-7 receptor upregulation on $CD8^+$ T cells. Moreover, HIV+ marijuana-users (MJ+) have fewer circulating $CD16^+$ monocytes compared with HIV+MJ-. In addition, monocytes and $CD8^+$ T cells, when cocultured with astrocytes significantly drive the secretion of astrocyte-derived inflammatory mediators, IL-6 and IP-10, a response suppressed by THC. Collectively these results suggesting that marijuana use may be protective against HAND.

S 2456 Resolution of Inflammation in Chemical Toxicity/Tissue Injury: What's Emerging?

K. Gowdy. *East Carolina University, Greenville, NC.*

Inflammation is a rapid and dynamic protective response to injury or infection. It involves a sequential accumulation of phagocytic neutrophils and macrophages derived from blood and bone marrow precursors at sites of injury/infection. The goal of the inflammatory response is to rid the body of pathogens, foreign materials, dead and dying cells, and debris and to restore normal tissue structure and function. Complete resolution of the inflammatory response and return to homeostasis are essential for restoring healthy tissues. Increasing evidence suggests that resolution is an active process that involves the downregulation of inflammatory cell recruitment and activation and the upregulation of cellular processes that drive tissue restoration. Recruitment of inflammatory cells and production of mediators have been implicated in the toxicity of diverse xenobiotics, whereas few have considered the alterations in the resolution process. In a number of experimental models, the pathogenic response to xenobiotics is a consequence of an impaired resolution of inflammation. Additionally, therapeutics that promote the resolution of inflammation response may limit xenobiotic-induced tissue injury and the development of chronic disease. The goal of this Symposium is to present new and emerging information on processes that regulate the resolution of inflammation and the how toxicant exposure alters these pathways. The first three speakers will focus on inflammatory macrophages as key cellular mediators of xenobiotic-induced pulmonary injury and the role of lipid mediators in regulating their ability to downregulate inflammation and initiate tissue resolution. The first talk will cover how xenobiotic exposure impairs macrophage clearance of apoptotic cells, termed *efferocytosis*, and its restoration by specialized pro-resolving mediators (SPMs), while the second talk will present on the role of lipid related transcription factors (FXR, PPARg) and downstream lipid transport genes (CD36, ApoE, ABCA1) as regulators of macrophage anti-inflammatory/wound repair activity in lung injury. The third talk will specifically focus on oxidized lipids and nitric oxide as regulators of necro-inflammatory cell death pathways in macrophages, including ferroptosis during inflammatory resolution. The next two talks will present the latest experimental and clinical approaches that are being tested for preventing and resolving toxicant-induced inflammation and injury in the lung; these include endogenous epoxyfatty acids, SPMs, and nitro-fatty acids. From this Symposium, the audience will gain knowledge about tools and technologies for assessing inflammation resolution, translational potential of intervention strategies, and research gaps and needs.

S 2457 Evolving Concepts in Inflammation Resolution and Future Opportunities

S. Naddadur. *NIEHS, Research Triangle Park, NC.*

This presentation will focus on the molecular mechanisms known to restore tissue homeostasis. There are critical knowledge gaps in our understanding of how toxicants/chemicals alter these processes that need to be investigated to improve the efficacy of clinical interventions.

S 2458 Mechanisms Mediating Perturbations in Alveolar Macrophage Efferocytosis Following Exposure to Toxicants

K. Gowdy. *East Carolina University, Greenville, NC.*

The lung is constantly exposed to inhaled toxicants, including air particulates and oxidant gases that trigger pulmonary inflammation. These insults can compromise gas exchange and induce irreversible tissue injury. Alveolar macrophages, which constitute approximately 95% of the immune cells found in murine and human lungs at homeostasis, are critical in regulating pulmonary inflammation after environmental insults. While it is well established that alveolar macrophages play an essential role in host defense by eliminating pathogens via phagocytosis, recently it has been shown that they also promote tissue homeostasis through efferocytosis, a process whereby macrophages engulf and eliminate apoptotic cells. Efferocytosis also produces anti-inflammatory mediators, such as IL-10, TGF-ß, and PGE2. This is essential to the resolution of pulmonary inflammation, the prevention of secondary necrosis, and the restoration of tissue homeostasis. A number of studies have linked impaired efferocytosis with chronic lung diseases, including asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis. Exposure to inhaled toxicants can specifically increase alveolar macrophage release of proinflammatory lipid mediators; however, whether toxicants alter the efferocytotic response of alveolar macrophages is unknown. Therefore, identifying the consequences of exposure to inhaled toxicants on efferocytosis and the molecular mechanism underlying potential defects will provide

key information related to how toxicants modulate the resolution of inflammation. This presentation will highlight the effects of inhaled toxicants on alveolar macrophage efferocytosis. Currently, an avenue of research that we are pursuing is investigating pulmonary lipid metabolism after ozone exposure, with emphasis on the production of specialized pro-resolving lipid mediators. Our data indicate that ozone exposure suppresses the pulmonary production of specialized pro-resolving lipid mediators, leading to decreased alveolar macrophage efferocytosis. These data, together with studies by other laboratories, link immune cell function with lipid metabolism to provide mechanistic insights for potential therapeutic interventions.

S 2459 Macrophage Phenotype and Inflammation in Lung Injury and Resolution: Role of Lipid Metabolism

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Macrophages play a key role in both the initiation of inflammatory responses and the resolution of inflammation and tissue repair. These diverse activities have been attributed to proinflammatory M1 macrophages and anti-inflammatory/wound repair M2 macrophages, which sequentially appear in injured tissues. Key to effective tissue repair is a balance in the activities of M1 and M2 macrophage subpopulations. Thus, whereas overactivation of M1 macrophages or underactivation of M2 macrophages can lead to exacerbation of tissue injury, excessive activation of M2 macrophages can result in chronic inflammation and fibrosis. We have been investigating the role of macrophages in lung injury induced by inhaled toxicants. In previous studies, we showed that both M1 and M2 macrophage subpopulations accumulate in the lung following exposure to pulmonary toxicants such as ozone, particulates, and mustard vesicants. We speculate that toxicant-induced lung injury and disease pathogenesis is due to a failure of M2 macrophages to suppress proinflammatory responses of M1 macrophages. RNA-seq analysis revealed that nuclear transcription factors involved in lipid handling, including PPARγ, LXR, and FXR, are downregulated in the lung after pulmonary toxicant exposure. This is associated with prolonged inflammatory activity of M1 macrophages and reduced anti-inflammatory activity of M2 macrophages. PPARγ agonists induced M2 macrophage activation and reduced lung inflammation and oxidative stress induced by pulmonary toxicants. Conversely, mice deficient in FXR exhibited exacerbated proinflammatory macrophage activity and prolonged oxidative stress. Taken together, these data indicate that lipid handling and metabolism are key to regulating macrophage activation and the resolution of inflammation following lung injury.

S 2460 Regulation of Macrophage Programmed Death in Inflammation by Redox Lipid Signaling

V. Kagan. *University of Pittsburgh, Pittsburgh, PA.* Sponsor: K. Gowdy

Signaling by macrophages orchestrates, to a large extent, the transition from proinflammatory responses to successful resolution of inflammation. Among these, the potent regulators of such signals are orderly dying apoptotic cells using oxidized phospholipids, phosphatidylethanol-amine, and cardiolipin, for this purpose. In contrast, chaotic signaling by necrotic materials enhances and perpetuates inflammation. Recent discoveries of necrotic cell death programs, particularly necroptosis, pyroptosis, and ferroptosis, point to their necro-inflammatory effects, thus emphasizing the important role of the type of cell death program in regulation of inflammation. Ferroptosis, the latest addition to the list of regulated necrosis, occurs due to the loss of control over three homeostatic mechanisms regulating iron, lipid peroxidation, and thiols. By applying redox lipidomics, we discovered that 15-lipoxygenase (15-LOX) complexed with phosphatidyl-ethanolamine binding protein 1 (PEBP1) catalyzes the generation of pro-ferroptotic signals, 15-hydroperoxy-eicosatetraenoyl-phosphatidylethanolamine (15-HpETE-PE), hence modulates the ferroptotic endurance. We further established that this pro-ferroptotic signaling is essential for the execution of the ferroptotic program not only by mammalian cells, but by prokaryotes, particularly bacterial pathogens (*P. aeruginosa*) as a "theft-ferroptosis" mechanism to manipulate the host inflammatory responses. Analysis of cell death programs in macrophages revealed that their characteristic phenotypic features define their susceptibility or resistance to ferroptosis. We discovered that iNOS/NO· is the major regulator of macrophage ferroptotic pathways and established the underlying molecular mechanisms. These new concepts of cell death-type propagated necro-inflammation versus non-necrotic apoptosis-driven resolution of inflammation will be discussed in the context of the essential lipid redox signaling.

S 2461 Endogenous Epoxyfatty Acids Prevent and Resolve Inflammation by Stabilizing Mitochondria and Reducing Pathological Endoplasmic Reticulum Stress

C. Morisseau. *University of California Davis, Davis, CA.* Sponsor: K. Gowdy

Lung inflammation is involved in a variety of pulmonary diseases, as well as following exposure to dust, environmental chemicals, and potential chemical warfare agents. The arachidonic acid cascade has long been known to play a role in pulmonary inflammation through two predominantly inflammatory branches driven by cyclooxygenase and lipoxygenase enzymes. Therapeutically, many drugs that reduce pulmonary inflammation either block the formation of inflammatory products from the cyclooxygenase and lipoxygenase branches of the arachidonic acid cascade or by blocking the action of these products. However, there is a third branch to the cascade known as the cytochrome P450 cascade, which is predominantly, but not exclusively, anti-inflammatory. Within this cascade, cytochrome P450 enzymes produce epoxides of polyunsaturated fatty acids, which act as powerful chemical mediators to stabilize against mitochondrial dysfunction, thus reducing reactive oxygen species and endoplasmic reticulum stress leading to inflammation and fibrosis. These epoxyfatty acid (EpFA) chemical mediators are largely degraded by an enzyme known as the soluble epoxide hydrolase (sEH), which works near the diffusion controlled limit to convert the EpFA to the corresponding 1,2-diols, which are either inactive or, in some cases, proinflammatory. Thus, inhibiting sEH increases inflammation resolving EpFA, which mitigates some pulmonary disorders. The sEH inhibitors in many cases are more active in animals with an ω-3 enhanced and an ω-6 depleted diet. They also synergize with FLAP inhibitors in asthma models. The sEH inhibitors have been effective in a variety of animal models of pulmonary inflammation, including asthma, COPD, and idiopathic pulmonary fibrosis, suggesting that sEH inhibitors, EpFA, or their mimics could be therapeutic agents. This is being explored in several human trials with COPD as a target.

S 2462 The Road from Bench to Bedside: the Clinical Development of the Nitro Fatty Acid CXA-10

D. Jorkasky. *Complexa, Berwyn, PA.* Sponsor: K. Gowdy

The evolution of an endogenous mediator with promising signaling actions into a regulatory approved pharmaceutical product requires extensive preclinical and clinical development. CXA-10 is the synthetic pure regioisomer, 10-nitro-octadec-9-enoic acid, of endogenous nitrated oleic acid (NO2 -OA), a prototypical molecule of a class of endogenously generated mediators called nitrated fatty acids (NO2 -FA). *In vitro* observations and *in vivo* animal models have demonstrated their significant cytoprotective effects through anti-inflammatory, immunomodulatory, and pro-metabolic signaling responses, which have pharmacological potential in human inflammatory diseases. To support human development, preclinical characterization of CXA-10 included the determination of its absorption, distribution, metabolism, and excretion; safety evaluation at doses greatly exceeding pharmacological activity; and development of validated assays for measurement of CXA-10 and its key metabolites. Dose/exposure response ranges of pharmacological activity were constructed to facilitate translation of CXA-10's pharmacokinetic/pharmacodynamic results into humans. Both IV and oral CXA-10 formulations were developed and utilized in phase 1 studies in lean and obese healthy volunteers and subjects with impaired kidney function. The mechanisms of NO2 -FA signaling mediated by CXA-10 observed in preclinical studies (heat shock response [HSR] and Nrf2 activation and NF-κB inhibition) have been translated at predicted dose/exposures in humans. Measurement of gene expression in peripheral blood mononuclear cells and urinary exosomes derived from the kidney demonstrated Nrf2 and HSR activation after a single dose, and significant inhibition of inflammatory mediators MCP-1, leptin, and IL-6 was demonstrated after multiple days of once daily oral dosing. The pro-metabolic effects of CXA-10 were observed by significant reductions in serum triglyceride and cholesterol levels. CXA-10's actions on Nrf2, which affects metabolic transporters, also were confirmed in a drug-drug interaction study. Overall, despite CXA-10's unique chemical pedigree as a reversible covalent electrophilic fatty acid with pluripotent pharmacological activity, human phase 1 studies have demonstrated the safety, tolerability, and predicted pharmacological actions of CXA-10 with once-a-day dosing.

2463 Single-Cell Technologies: A Potentially Transformative Tool for Toxicology

S. Bhattacharya. *Michigan State University, East Lansing, MI.*

Single cell genomics and epigenomics have been among the breakthrough biological technologies of the last few years. These methods provide detailed views of living systems at unprecedented resolution and bypass the problems associated with averaging over heterogeneous cellular responses in bulk assays. Some spectacular successes of single cell RNA sequencing (scRNA-seq) include cell-by-cell reconstruction of transcriptomic atlases of xenopus and zebrafish embryos, along with ambitious undertakings like the Human Cell Atlas to map the transcriptomes of all cell types in the human body. This timely and topical Symposium will provide an overview of the capacities and limitations of current single cell technologies and describe cutting-edge applications of single cell methods in detecting perturbations in cellular state in health and disease, including identification of rare and altered cell types. The presenters will explore the role of single cell transcriptomic profiling in revealing phenotypic heterogeneity among breast cancer stem cells, mapping alterations in the developmental trajectory of early germ cells subject to environmental insult, discovery of a novel subpopulation of alveolar macrophages in an animal model of asbestos-induced pulmonary fibrosis, and identification of an altered effector CD8 T lymphocyte population in smokers. Overall, the presentations in this Symposium will introduce the field of single cell 'omics and provide a snapshot of the first generation of applications of these novel technologies in toxicology.

2464 Dissecting Complex Systems with Multidimensional Single Cell Data

G. Zheng. *Arsenal Biosciences, South San Francisco, CA.* Sponsor: S. Bhattacharya

Biological systems are complex, with many individual components. Discriminating the subunits and their relationships is critical to elucidating biological systems and gaining mechanistic insights from genotypes to phenotypes. In the last few years, advances in single cell technologies have made it possible to study intercellular relationships among complex tissues at unprecedented scale, resolution, and precision. These advances include the ability to query the genome, transcriptome, epigenome, and proteome in a large number of cells at single cell resolution and the ability to perturb the genome and epigenome of every single cell. This presentation will describe single cell technologies in each area and the expanding number of available experimental platforms, along with key examples of applications that have already advanced our understanding of biology. In particular, the power of profiling gene expression and a panel of cell surface proteins from the same cells, and recent efforts to use this technology to discover T cell receptor and antigen interaction at scale, will be discussed. In addition, a case study of single cell epigenetic profiling of intratumoral T cells from solid tumor biopsies will be presented. The presentation will close with a discussion of trends in single cell 'omics research over the next few years.

2465 Single Cell Profiling to Characterize Breast Stem Cell Heterogeneity in Development and Cancer

J. A. Colacino. *University of Michigan, Ann Arbor, MI.*

Emerging epidemiological evidence points to the number of stem cells in a tissue as being a key determinant of risk of multiple cancers, including breast cancer. Breast cancers also are driven and sustained by a stem-like population of cells. Breast cancer stem cells transit between multiple phenotypic states, from mesenchymal-like to epithelial-like states. Stem cell heterogeneity in the normal human breast is not well understood but could be an important predictor of cancer risk. To better understand this heterogeneity, we profiled normal reduction mammoplasty tissues by flow cytometry for two known markers of breast stem cells, ALDH+ and CD44+/CD24-. RNA-seq of sorted ALDH+ cells revealed an epithelial phenotype, reflected by expression of CDH1, EPCAM, and KRT8. CD44+/CD24- had a mesenchymal expression pattern, reflected by high VIM, CDH2, and ZEB2 expression. Isolated ALDH+ normal breast stem cells were profiled for single cell gene expression using the Fluidigm C1 and Biomark systems. Unbiased hierarchical clustering revealed that the ALDH+ single cells clustered in three classes, an epithelial-like, a mesenchymal-like, and a hybrid epithelial/mesenchymal (E/M) phenotype. Hybrid E/M cells also expressed high levels of genes associated with poor prognosis in triple negative breast cancer samples, including CD146, KRT7, and NOTCH3. Additionally, we used drop-seq for single cell RNA-seq on normal mammary epithelial cells grown in conditional reprogramming (CR) conditions. These cells were enriched in hybrid E/M cells, relative to mammary epithelial cells, which were not grown in CR. Aligning the CR cells to publicly available single cell RNA-seq data generated by sampling mouse mammary gland at multiple time points through the life course shows that hybrid E/M cells preferentially map to the *in utero* mammary gland. Thus, the hybrid E/M state reflects an *in utero* stem cell state, with gene expression patterns activated in aggressive breast cancers. Ongoing work is using single cell profiling of breast epithelial cells from African American women and European American women to test the hypothesis that the increased risk of triple negative breast cancer in African American women is linked to an expansion of hybrid E/M stem cells and testing whether toxicant exposures can cause expansion of this rare cell population.

2466 Understanding Environmental Impact on Germ Cell Differentiation Trajectories Using scRNA-Seq

P. Allard. *University of California Los Angeles, Los Angeles, CA.*

While the intricate steps of germ cell development have long been hypothesized to provide unique windows of sensitivity to environmental insults, little is known of the effect of chemical exposure on the epigenome of germ cells and of the mechanisms of inheritance of these effects. This gap is particularly significant as embryonic germ cells undergo an extensive remodeling of their chromatin, which includes genome-wide demethylation and the establishment of a complex pattern of histone modifications. The failure to properly regulate these histone marks leads to spurious repetitive element expression, germ cell death, and infertility. Here, we use *in vitro* generated mouse and human germ cells termed *Primordial Germ Cell-Like Cells* (PGCLCs) to conclusively show that their epigenetic remodeling period constitutes a period of high sensitivity to environmental exposure. To this aim, we exposed their precursors, epiblast-like cells (EpiLCs) as well as PGCLCs for various lengths of time to (1) known chemical inhibitors of specific histone marks, and (2) a model environmental chemical: BPA. We examined the impact of these exposures on survival/viability as well as the levels of repressive histone marks such as H3K9me3 and H3K27me3. By using targeted chemical inhibitors, we show that the tight regulation of both repressive histone marks, H3K9me3 and H3K27me3, is essential for survival of differentiating PGCLCs. We also show that low BPA concentrations (1 and 10 uM) disproportionately affect PGCLCs survival but not EpiLCs. These effects correlate with a marked decrease of both repressive marks, H3K9me3 and H3K27me3. Finally, by using scRNA-seq, we show that exposure of human PGCLCs to low BPA levels doses alters the developmental trajectory of PGCLCs and affects precursors of PGCLCs disproportionately compared with PGCLCs themselves. We expect this research to provide a much-needed examination of the pathways implicated in the sensitivity of early germ cells to environmental insults and at the root of infertility.

2467 Single Cell Transcriptomics Identifies Key Cellular Players in an Animal Model of Asbestos-Induced Pulmonary Fibrosis

N. Joshi. *Northwestern University, Chicago, IL.*

Occupational and indoor exposure to asbestos can lead to development of pulmonary fibrosis years after exposure, leading to significant morbidity and mortality. Asbestos fibers can lodge within and persist in the bronchoalveolar duct junctions and small airways of humans and mice, respectively. Ontologically distinct populations of macrophages can contribute to asbestos-induced lung fibrosis. Using a comprehensive combination of unbiased single cell transcriptomic profiling (scRNA-seq), genetic lineage tracing, flow cytometry, and *in situ* RNA hybridization, we tested the hypothesis that monocyte-derived alveolar macrophages are key drivers of asbestos-induced pulmonary fibrosis. C57Bl6 mice were exposed to TiO2 (control) or asbestos fibers intratracheally. Lungs were harvested to capture the early stages of pulmonary fibrosis, and scRNA-seq libraries were prepared from cell suspensions using the 10X Chromium platform. Profiling 24,060 cells identified 24 known cellular populations represented in all experimental conditions. All populations exhibited transcriptional changes during the development of fibrosis. Importantly, the emergence of a new, distinct subpopulation of alveolar macrophages was observed in asbestos-exposed animals. This subpopulation was characterized by an immature phenotype and elevated expression of genes known to be causally associated with fibrosis, such as Mmp12, Retnla, Chia1, and Pdgfa (involved in fibroblast proliferation). Furthermore, these cells expressed Itgam and Cx3cr1, suggesting a monocyte origin. Remarkably, this new subpopulation was represented only by cells from asbestos-exposed mice and was absent in control conditions. Flow cytometry, lineage-tracing analyses, and immunohistochemistry confirmed this subpopulation to be monocyte-derived alveolar macrophages. Immunofluorescent microscopy confirmed that Pdgfa-expressing cells were specifically recruited to the areas of fibrosis and were located in the proximity of Pdgfra-expressing fibroblasts.

Cre/lox-mediated genetic deletion of this population by targeting Casp8 prevented the development of pulmonary fibrosis. Collectively, these studies are the first to show a causal association between asbestos-induced epithelial lung injury, localized recruitment of monocyte-derived alveolar macrophages, and subsequent development of spatially restricted lung fibrosis.

2468 Single Cell RNA Sequencing Reveals Altered Effector CD8 T Lymphocytes in Smokers

S. N. Martos. *NIEHS, Research Triangle Park, NC.*

As a risk factor for human diseases, the global disease burden attributed to tobacco smoke exposure remains substantial. In addition to DNA damage, smoking alters the epigenome and transcriptome of human blood leukocytes. Interpretation of bulk genomic approaches is limited because changes could indicate altered distribution of cell (sub)populations or changes in expression within (sub)populations. To characterize smoking-related gene expression changes in primary immune cells, we performed single cell RNA sequencing (scRNA-seq) on >45,000 human peripheral blood mononuclear cells (PBMCs) from smokers (n = 4) and nonsmokers (n = 4). Transcriptomes revealed an altered subpopulation of Natural Killer (NK)-like T lymphocytes in smokers, which expressed elevated levels of FCGR3A/CD16 compared with other CD8 T cell subpopulations. Relatively rare in nonsmokers (median: 1.8%), the transcriptionally unique subset of CD8 T cells comprised 7.3% of PBMCs in smokers. Among CD8 T cells, the increase in NK-like cells (Mann-Whitney p = 0.03) corresponded with a decrease in naïve cells (p = 0.03). Mass cytometry (CyTOF; >1 million cells) of 26 cell surface marker proteins confirmed an increase in CD16+ CD8 T cells in smokers. We did not observe differences in the overall frequencies of CD8 T cells or NKT cells between smokers and nonsmokers by either scRNAseq or mass cytometry. This indicates that smoking is associated with an increased number of CD8 T cells that share characteristics with NK cells, but are not NKT cells. Consistent with an NK-like phenotype, altered effector CD8 T cells had elevated expression of genes reported to be upregulated in T cells reprogrammed to NK-like cells. Compared with other effector CD8 T cells, altered effector CD8 T cells had reduced IL7R and increased IFNG (interferon γ), GZMB (granzyme B), and PRF1 (perforin) expression. In mice, granzyme B and perforin expressing CD8 T cells contribute to the development of atherosclerotic plaques. Our data highlight a potential link between smoking-induced functional changes in human CD8 T cells and atherosclerosis.

2469 Applying Modern Toxicology to Dietary Supplements with Ancient Roots: Botanical Safety in the 21st Century

C. Rider. *NIEHS/NTP, Research Triangle Park, NC.*

Botanical dietary supplements are consumed by millions of people globally, with tens of thousands of products available in the US marketplace. Ensuring the safety of these products is an important public health priority. However, evaluating their safety can be challenging due to the inherent complexity and significant variability in composition and quality, including the possibility of contamination and adulteration. Furthermore, the bioactive constituents in botanicals are often unknown, necessitating toxicological evaluation of the whole mixture. While there have been significant advancements in the development of new approach methodologies (NAMs) for read-across and screening/prioritization of single chemicals, there has been much less attention focused on adapting and evaluating these methods for application to complex mixtures. Botanical dietary supplements offer a unique opportunity for exploring the extension of NAMs to complex mixtures and building confidence in their application to a risk evaluation context. Toward this goal, there are several key areas that require attention. First, it is necessary to understand the current regulatory landscape for botanicals (i.e., What is the historical context and what are the current approaches being used to ensure product safety?). Next, an overarching challenge in evaluating botanicals is their complex chemistry. Therefore, defining the level and nature of non-targeted and targeted chemical analysis necessary for complex mixture characterization is critical. In terms of hazard characterization, identifying the critical endpoints and assays to include for safety evaluation is an important first step. This effort requires identifying likely biological targets of botanical toxicity (e.g., toxicity signals in adverse event reporting and animal studies) and evaluation of fit-for-purpose NAMs. Finally, to build confidence in any NAM-based safety assessment, it is critical to build orthogonal datasets to compare NAM results with toxicity observed in animal models and humans. Research efforts in each of these key areas will be highlighted by speakers with diverse perspectives.

2470 Applying Modern Toxicology to Botanical Dietary Supplements

M. Embry. *HESI, Washington, DC.*

The 1994 Dietary Supplement Health and Education Act provides the statutory basis for regulating the safety of botanical dietary supplements. Historically, regulatory guidance in the United States has maintained a botanical safety framework that relies on history of safe use and traditional animal testing paradigms with virtually no utilization of *in vitro* and *in silico* approaches. Recently, the US Food and Drud Administration's *Predictive Toxicology Roadmap* described the Agency's dedication to fostering the development and evaluation of new and emerging toxicological tools and technologies for incorporation into regulatory review. The Botanical Safety Consortium (BSC) is a newly formed public-private partnership that embodies the spirit of applying predictive toxicological tools to evaluation of botanical dietary supplement safety. The BSC is a collaboration between scientists in industry, government, and academia, formed with the goal of providing a sound scientific basis for integrating existing data with the latest toxicology tools to evaluate botanical safety. Chemical characterization of complex botanical products and identification of fit-for-purpose assays for evaluating genotoxicity, hepatotoxicity, developmental and reproductive toxicity, cardiotoxicity, and repeat-dose systemic toxicity are key areas to be explored by the consortium.

2471 Chemical Analysis: The Foundation of Botanical Safety

J. Kellogg. *Pennsylvania State University, State College, PA.* Sponsor: C. Rider

Chemical characterization is a critical "first step" in the evaluation of botanical dietary supplement safety. Principally, results from botanical dietary supplement testing cannot be interpreted without precise knowledge of the test substance (i.e., identity of the ingredients, authenticity, quality). There are numerous methods that can be used for this purpose, including DNA barcoding and various analytical chemistry methods (e.g., HP-TLC, HPLC, NMR), each with advantages and disadvantages. Regardless of the specific approach employed, the resulting data are typically high-dimensional, requiring chemometric analysis for interpretation. Application of these approaches will be presented using green tea and goldenseal root extract examples. The green tea example will illustrate the decision tree involved in test article selection based on comparison across numerous related products. The goldenseal example focuses on determination of authenticity and identification of adulterated samples.

2472 Developing and Applying *In Vitro* Liver Models to Address Potential Hepatotoxicity of Botanical Dietary Supplements

A. Roe. *Procter & Gamble, Cincinnati, OH.*

The liver has been identified as an important target for potential injury from botanical dietary supplement exposure. Oral administration to relatively high doses (100s to 1000s mg per day) of botanicals predisposes the liver to insult. Furthermore, the Drug-Induced Liver Injury Network (DILIN) has identified bodybuilding supplements as well as other categories of dietary supplements with increasing incidents of liver injury. Examples of botanical products that have been associated with significant liver toxicity include green tea extract, OxyElite Pro, and Hydroxycut. However, due to the complex chemistry of botanicals, it has been difficult to identify the botanical ingredient or specific constituent associated with observed liver toxicity. The liver has been an area of innovation for development of *in vitro* and *in silico* tools and technologies for evaluating toxicity due to its critical role in drug metabolism and, relatedly, safety testing. Fortunately, holistic liver models that express sufficient baseline functionality for uptake and efflux transporters, metabolizing enzymes, and the regulatory pathways for both exist for the liver. In fact, these types of liver models have been shown to be very useful in studying complex botanical mixtures. Examples of the application of sandwich-cultured human hepatocytes to predict clinically relevant botanical-drug clearance interactions associated with *Schisandra spp*., St. John's wort, and *Boswellia serrata* will be discussed.

2473 Systems Toxicology Approaches to Evaluate Botanical Safety

J. Dever. *Amway Nutrilite, Grand Rapids, MI.*

Developing *in vitro* models that incorporate interactions between organ systems and reflect the complexity of biological systems is required to move beyond animal testing. The goals of systems toxicology work in safety evaluation of botanicals are twofold: (1) to build models that allow for higher-throughput assessment of botanicals in complex biological systems, and (2) to generate data allowing for translation from *in vitro* systems to human and animal model contexts (i.e., *in vitro* to *in vivo* extrapolation; IVIVE). Toward the first goal, model systems that have the capacity to recapitulate flow between cell-based organ models are currently under development. Initial testing with botanical dietary supplements will be presented. Regarding the second goal, examples of predicting *in vivo* concentrations based on *in vitro* data will be discussed.

2474 Challenges and Approaches for Using Animal and Human Data to Evaluate *In Vitro* Systems in Botanical Safety Assessment

C. Rider. *NIEHS/NTP, Research Triangle Park, NC.*

Building confidence in new approach methodologies (e.g., *in vitro* and alternative approaches) requires the development of case studies that include orthogonal data from traditional sources (i.e., animal toxicity studies, epidemiological studies, and adverse event reports) to define their potential application and limitations. The National Toxicology Program (NTP) has evaluated the toxicity and carcinogenicity of multiple widely used botanical dietary supplements (e.g., goldenseal root powder, kava kava, green tea extract) in mice and rats. Studies have ranged from chronic and comprehensive evaluation of toxicity for hazard identification to targeted studies aimed at better understanding dose-response for a known toxicity (e.g., cardiotoxicity of ma huang with and without caffeine). A key question is how to use this body of animal research combined with human data from epidemiological studies and adverse event reporting to evaluate the utility of *in vitro* systems in a botanical safety assessment context. Challenges include accounting for discordant data, extrapolating across species, and identifying the level of mechanistic data required to support conclusions. Case studies for botanical dietary supplements (e.g., *Ginkgo biloba* extract, green tea extract) that include both animal and human data will be compared with available *in vitro* data to illustrate the challenges and opportunities moving forward.

2475 Automation and Machine Learning Techniques to Leverage Resources When Conducting a Systematic Review

V. Walker. *NIEHS/NTP, Research Triangle Park, NC.*

Systematic review is a comprehensive, objective, and transparent multistep process to identify, screen, and synthesize primary scientific literature for reaching evidence-based hazard conclusions on specific research questions. Across the field of toxicology, efforts to apply systematic review methods are gaining momentum, highlighted by regulatory requirements that have been instituted globally to conduct systematic review in support of safety assessments of chemicals and foods (e.g., the US Environmental Protection Agency [US EPA] is required to use systematic review methods under the Toxic Substances Control Act [TSCA]). The National Toxicology Program (NTP), US EPA Integrated Risk Information System (IRIS), and European Food Safety Authority (EFSA) have all implemented systematic review methods in the conduct of either hazard assessments or risk assessments. As highlighted by the systematic review exposure summit in April of 2019, there are efforts to take advantage of the increased transparency and rigor of systematic review methods and apply these techniques to other disciplines of toxicology. However, the principal barrier to greater adoption and innovation of systematic review methods is the level of effort required in an area of fast-paced research and publication. Conducting a systematic review is time-consuming and resource intensive. Effort depends on the size of the evidence base, but on average, systematic reviews can take more than 1,000 hours to complete and can cost over $100,000 USD. This cost varies according to the number of questions being addressed and the amount of literature to evaluate. Further, by the time a systematic review is published, those data can be outdated, and thus perhaps not useful for rapid decision-making. Many of the steps of the systematic review process have the potential to utilize automated or semiautomated approaches; in fact, considerable progress has been made and additional work is underway to automate steps in the review process. Advances in natural language processing, text mining, and machine learning have produced new algorithms that assist with screening and significantly reduce the manual screening burden of large systematic reviews. Automation advances in the SR process require the skills and knowledge of a variety of experts, such as information specialists, developers, software engineers, statisticians, artificial intelligence experts, and researchers from various disciplines who conduct SRs. Given the increasing volume of published studies, automation and semiautomation of labor-intensive steps in the review process have great potential to improve the speed of systematic review and reduce the workload and resources required without compromising the rigor and transparency that are critical to the method. In this Workshop, speakers will introduce the concept of automation or semiautomation in the various steps of the systematic review process and highlight their utility and limitations in reducing the time and resources required to conduct a SR. The session will begin with an overview of basic systematic review principles and a brief description of why systematic review is valuable and impactful to environmental health risk assessments. In addition, a brief introduction to the methods involved in the process and discussion of various steps that are resource or time intensive will be presented. After the introduction, presentations will focus on the individual tasks of screening, data extraction, and risk of bias in the systematic review process and introduce current thoughts and approaches to developing and implementing automated or semiautomated tools to aid in the systematic review process. The final presentation will address the importance of generating training sets to develop gold standard corpora and performance benchmarks for future methods development and evaluation. Collectively, this Workshop will demonstrate current progress and challenges in development and implementation of automation and semiautomated tools and approaches for the labor-intensive tasks of the systematic review process. The Workshop will conclude with a panel discussion where the speakers, who represent industry, academia, and government, will discuss how current advancements and challenges can shape the future development of automation tools to support the systematic review process and impact the time, cost, and resource burden of conducting systematic reviews that support decision-making in environmental toxicology risk assessment and hazard identification.

2476 Brief Overview of Systematic Review Methodology and Identification of the Labor-Intensive Tasks Suitable for Automation Approaches

V. Walker. *NIEHS/NTP, Research Triangle Park, NC.*

Systematic review is rapidly becoming the gold standard for addressing environmental health questions. Across the field of toxicology, efforts to apply systematic review methods are gaining momentum, highlighted by regulatory requirements that have been instituted globally to conduct systematic review in support of safety assessments of chemicals and foods. NTP, US EPA IRIS, and EFSA have all implemented systematic review methods in the conduct of either hazard assessments or risk assessments. The systematic review process involves the objective and transparent method of collecting and synthesizing data for reaching hazard conclusions on specific research questions. This overview will provide a brief introduction to systematic review methodology and the impact of this approach to inform decision-making and will highlight steps in the process for which automation approaches could potentially be implemented to potentially reduce the cost and time necessary to conduct systematic reviews of environmental health questions.

2477 SWIFT-Active Screener: Accelerated Document Screening through Active Learning and Integrated Recall Estimation

R. Shah. *Sciome LLC, Durham, NC.*

In the screening phase of systematic review, researchers use detailed inclusion/exclusion criteria to decide whether each article in a set of candidate citations is relevant to the research question under consideration. A typical review may require screening thousands or tens of thousands of articles in this manner and require hundreds of person hours of labor. Here, we introduce SWIFT-Active Screener, a web-based, collaborative systematic review software application designed to reduce the overall screening burden required during this resource-intensive phase of the review process. Active Screener uses empirically validated statistical models designed to save screeners time and effort by automatically prioritizing articles as they are reviewed, using user feedback to push the most relevant articles to the top of the list. Meanwhile, a separate statistical model estimates the number of relevant articles remaining in the unscreened document list. Together, the combination of the two models allows users to find relevant documents sooner and provides them with accurate feedback about their progress. We evaluated the Active Screener document prioritization and recall estimation models using 26 systematic review datasets that were previously curated manually by reviewers. The results demonstrate that using Active Screener can result in significant time savings

compared with traditional screening and that the savings are increased for larger project sizes. For example, in document sets with 5,000 or more documents, the average WSS95 obtained is 61%. Furthermore, the recall estimator we have proposed provides a useful, conservative estimate of the percentage of relevant documents identified during the screening process. The software is currently available in the form of a multi-user, collaborative, online web application and has been used successfully to reduce the effort required to screen articles for systematic reviews conducted at a variety of organizations, including NIEHS, US EPA, USDA, TEDX, and EBTC. These early adopters have provided us with an abundance of useful data and user feedback, and we have identified several areas where we can continue to improve our methods and software. Several new features have been planned for the software, and it will be developed, improved, and maintained for the foreseeable future.

2478 Semiautomated Data Extraction to Reduce Extraction Time While Maintaining Quality and Reproducibility

Z. Lu. *NIH, Bethesda, MD.* Sponsor: V. Walker

Manually extracting relevant data from included studies is a critical yet rate-limiting step in conducting systematic reviews. Hence, facilitating this laborious and time-consuming step by automated approaches holds great potential to reduce the overall time required to complete a systematic review. In this presentation, an overview of recent developments in natural language processing (NLP) techniques (e.g., deep learning-based approaches) in biomedical concept recognition and information extraction will be presented, with a focus on data elements that are commonly sought in conducting systematic reviews. In addition, state-of-the-art NLP tools and current challenges and future opportunities in putting such tools into action will be discussed.

2479 Semiautomated Approaches to Assess Risk of Bias (Study Quality)

J. Liao. *University of Edinburgh, Edinburgh, United Kingdom.* Sponsor: V. Walker

Certain aspects of study design are associated with different estimates of biological effects, and this is important when assessing the credibility of research claims. Considering risks of bias in the systematic review process allows more sophisticated insights to the credibility of reported findings, both at individual (study) and topic (literature) levels. However, ascertaining risks of bias can be both time-consuming and subjective, and—if the ambitions of living systematic reviews are to be realized—tools to automate this process are required. These tools fall into two broad categories. In the first, a given string is identified (Dictionary approach), perhaps considering neighboring strings or other text features (Regular Expressions). In the second, machine-learning algorithms such as convoluted neural networks are trained on labeled learning manuscript sets. While this approach has shown value in ascertaining risks of bias in reports of clinical trials, its use in laboratory biomedical research is less well developed. Challenges include the size of the training datasets required, the risks of propagation of human error into machine performance, the lack of transparency in algorithm decision-making, and the need to convert manuscripts to simple text files. A RegEx for selected risks of bias is enabled on the free-to-use Systematic Review Facility (app.syrf.org.uk), and we are updating this for use in institutional research improvement activities. The early performance of our Convoluted Neural Network approaches that achieved by our RegEx.

2480 If You Can't Measure It, You Can't Improve It: On the Importance of Benchmark Datasets

A. Nowak. *Evidence Prime, Kraków, Poland.* Sponsor: V. Walker

One of the hurdles to adoption of systematic review automation tools is lack of good-quality datasets for the individual tasks (such as screening or data extraction). Such datasets are required both for measuring improvements (introduced by new research) and comparing strong and weak points of various systems to select the best tool for the job. They need to be large enough to produce sufficient precision of estimates. They also need to represent diverse use cases, in which systems are used in the wild. Moreover, their quality and collection methods need to reflect methodologies employed by prospective users. Satisfying all these requirements comes at significant cost, so it is in the best interest of the whole automation community to make these datasets reusable and shareable. As is the case in the other machine-learning research areas, benchmarks need to be established, preferably with public leaderboards (e.g., the EBM-NLP project from Northeastern University). This would enable objective comparison of work coming from different groups and contribute to more rapid advances in the field. The work required for creation of shared datasets, such as harmonizing data formats, also could pay off by bringing the community closer to achieving interoperability among the tools. A big investment toward construction of the datasets is building and maintaining the annotation tools. This cost could be shared among the groups by collaboration on the tools and releasing them on open-source licenses. This in turn would enable organizations to create their own application-specific benchmarks and evaluations of the available solutions.

2481 *In Vitro* Microphysiological Systems: Developing Tools to Evaluate Immunotoxicity of Drug Candidates

N. Marshall. *GlaxoSmithKline plc, Collegeville, PA.*

There is a crucial need for new technologies that can reliably predict drug safety and efficacy in humans in preclinical studies. Advances in bioengineering, material sciences, microfabrication, and microfluidics technologies have enabled the development of microphysiological systems that mimic the functional units of an organ. These systems recreate the specialized multicellular architectures, tissue-tissue interfaces, physicochemical microenvironments, and vascular perfusion necessary to *rec*Apitulate organ-level physiology *in vitro* and have emerged as key tools that permit the study of human physiology. The more recent development of biomimetic immune organs-on-chips, or systems incorporating immune components, increases physiological relevance of these models to drug development and can allow for bidirectional assessment of human immune cell and tissue interactions in a controllable microenvironment. An integrated immune competent microphysiological platform could further enhance our understanding of disease etiology and fill the critical need for improved preclinical model systems to predict efficacy, safety, bioavailability, and toxicity outcomes for candidate compounds. This session will highlight recent advances in system-on-chips models that predict immune-driven toxicities.

2482 Human Bone Marrow Dysfunction Recapitulated *In Vitro* Using Organ Chip Technology

D. Chou. *Wyss Institute for Biologically Inspired Engineering at Harvard University, Boston, MA.* Sponsor: N. Marshall

Understanding human bone marrow (BM) pathophysiology in the context of myelotoxic stress induced by drugs, radiation, or genetic mutations is of critical importance in clinical medicine. However, study of these dynamic cellular responses is hampered by the inaccessibility of living BM *in vivo*. Here, we describe a vascularized human Bone Marrow-on-a-Chip (BM Chip) microfluidic culture device for modeling bone marrow function and disease states. The BM Chip is composed of a fluidic channel filled with a fibrin gel in which patient-derived CD34+ cells and bone marrow-derived stromal cells (BMSCs) are co-cultured, which is separated by a porous membrane from a parallel fluidic channel lined by human vascular endothelium. When perfused with culture medium through the vascular channel, the BM Chip maintains human CD34+ cells and supports differentiation and maturation of multiple blood cell lineages over one month in culture. Moreover, it recapitulates human myeloerythroid injury responses to drugs and gamma radiation exposure, as well as key hematopoietic abnormalities found in patients with the genetic disorder Shwachman-Diamond syndrome (SDS). These data establish the BM Chip as a new human *in vitro* model with broad potential utility for studies of BM dysfunction.

2483 A Foreign Body Response-on-a-Chip Platform

A. Khademhosseini. *University of California Los Angeles, Los Angeles, CA.* Sponsor: N. Marshall

Understanding the foreign body response (FBR) and designing strategies to modulate such a response represent a grand challenge for implant devices and biomaterials. Here, the development of a microfluidic platform is reported (i.e., the FBR-on-a-chip [FBROC]) for modeling the cascade of events during immune cell response to implants. The platform models the native implant microenvironment where the implants are interfaced directly with surrounding tissues, as well as vasculature with circulating immune cells. The study demonstrates that the release of cytokines such as monocyte chemoattractant protein 1 (MCP-1) from the extracellular matrix (ECM)-like hydrogels in the bottom tissue chamber induces trans-endothelial migration of circulating monocytes in the vascular channel toward the hydrogels, thus mimicking

implant-induced inflammation. Data using patient-derived peripheral blood mononuclear cells further reveal inter-patient differences in FBR, highlighting the potential of this platform for monitoring FBR in a personalized manner. The prototype FBROC platform provides an enabling strategy to interrogate FBR on various implants, including biomaterials and engineered tissue constructs, in a physiologically relevant and individual-specific manner.

W 2484 Application of Immunocompetent Microphysiological Systems in Drug Development

R. Horland. *TissUse GmbH, Berlin, Germany.* Sponsor: N. Marshall

Microphysiological systems enabling long-term co-culture of various human organ equivalents have become increasingly useful for preclinical systemic drug testing. Until now, these systems lacked integration of systemic immunocompetence and therefore didn't generate human immune responses. This hampers their use for the evaluation of vaccines, adjuvants, and stem, somatic, and immune cell therapies as well as modern wound-healing materials, implants, and the investigation of mechanisms of organ rejection. Here, we provide examples of successful development of immunocompetent microphysiological systems. This includes the generation and long-term cultivation of human organoid lymphatic tissues in a Multi-Organ-Chip platform using a chip-compliant hydrogel matrix. Employing the model at a larger scale, it already enables long-term and repeated drug exposure to induce and monitor both cellular and humoral immune responses. Cellular immunity is monitored by cytokine release patterns, while humoral immunity is detected by B cell activation, plasma cell formation, and antibody secretion profiles. Moreover, cellular composition and micro-organoid formation are analyzed by flow cytometry, histology, and *in situ* imaging. Another example is a skin allograft-on-a-chip model. Here allogenic PBMC and skin were co-cultured in a Multi-Organ-Chip system. PBMCs could be tracked in the skin over nine days while first signs of rejection could be detected by increased levels of IFN-gamma, IL-2, and IL-17A. Furthermore, the model may also be adapted to investigate pathogenic mechanisms in chronic inflammatory diseases (e.g., psioriasis).

W 2485 *In Vitro* Microphysiological Systems: Advancing Regulatory Science through Innovation

S. Fitzpatrick. *US FDA, College Park, MD.*

Novel methods such as *in vitro* microphysiological systems or organs-on-a-chip—microfluidic biomimetic devices that aspire to emulate the biology of human tissues, organs, and circulation *in vitro*—are enabling a global paradigm shift in regulatory science. Organ-on-a-chip devices are designed as accurate models of the structure and function of human organs, such as the lungs, liver, and heart. Once developed and integrated, researchers and regulators can use these models to predict whether a candidate drug, vaccine, or biologic agent is safe or toxic in humans in a faster and more effective way than current animal methods. Beyond use in drug research, organ-on-a-chip models will be used as test systems for development of non-pharma treatment approaches, like radiotherapy or hyperthermia for cancer, but also to develop new cosmetics or food additives and for toxicology analysis of environmental contaminants.

W 2486 Sex, Lungs, and Air Pollution

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The National Institutes of Health (NIH) recently mandated that sex be considered a biologic variable in animal and clinical research. Recent focus on sex-dependent biologic effects in toxicology has translated into new discoveries of sex differences in pulmonary responses to respiratory toxicant exposures, such as tobacco and wildfire smoke, ozone, and ambient air pollution. As the effects span multiple models, it suggests common mechanisms that should be highlighted and considered when evaluating respiratory toxicant exposure. This session will explore recent innovative research on sex-dependent pulmonary effects of air pollution. The first speaker will introduce the biology behind the development of respiratory sex differences and its importance for regulatory and safety considerations. Differences in susceptibility to disease, morbidity, and mortality related to respiratory sex differences will be described. For example, asthma prevalence rates are higher in boys than in girls, but this pattern reverses at the onset of puberty, and reverses again following the onset of menopause. The speaker will also review sex differences in nonclinical respiratory toxicology research models and how they contribute to regulatory decisions regarding human safety and risk assessment. The next talk will discuss the impact of *in utero* secondhand smoke (SHS) exposure on the development of cancers later in life and their sex dependence. Though it is well established that SHS exposure contributes to lung disease-related deaths, the onset and progression demonstrates sex specificity. This speaker will illuminate possible mechanisms, including genetic or hormonal influences, for these sex-specific responses using a rodent model of SHS exposure and urethane-induced tumorigenesis. Overall, SHS exposure *in utero* promotes metastasis in females through matrix remodeling, while promoting large tumors in males. The third speaker will describe sex-specific patterns of inflammation and airway hyperresponsiveness (AHR) in ozone exposure that impair lung innate immunity. The findings suggest that fluctuations of circulating hormone levels affect ozone-induced inflammatory responses. In this study, male and female mice underwent gonadectomy and hormone replacement prior to ozone exposure. After exposure, male mice displayed higher AHR than controls, while in females, AHR was reduced, both of which were ameliorated by hormone replacement. Together, these results indicate that sex hormones can modulate ozone-induced inflammation and AHR in adult mice. The fourth speaker will discuss how wood and biomass smoke impacts respiratory and immune function in primates across the life span. Wildfire events are a growing public health concern with the potential to increase cardiac and respiratory disease burden. However, little is known about the long-term effects of acute wildfire smoke inhalation. The speaker will report on recent research on the health of ambient wildfire smoke-exposed primates. Sex-specific responses in lung and immune function throughout the primate life span were observed. At infancy, females showed reduced lung compliance, which persisted into adulthood. These findings suggest that exposure age and sex enhance vulnerability to long-term health outcomes following exposure to wildfire smoke.The fifth speaker will describe how *in utero* exposure to ambient air pollutants in combination with prenatal stress can induce sex-specific respiratory outcomes in epidemiologic studies. Using epidemiological data, the speaker will describe how prenatal ambient pollution exposure and stress can sex-specifically modify children's respiratory outcomes. The speaker also will identify critical windows of developmental exposure, which result in adverse respiratory outcomes. This highlights that in humans, similar to other model systems, there are sex-specific windows of vulnerability and adverse outcomes of exposure to respiratory toxicants. Finally, there will be a scheduled panel discussion on how sex-specific findings in respiratory toxicant exposures influence funding, risk and safety assessments, and standard setting. This discussion will help define best practices for inclusion of sex as a biological variable in toxicity testing from cell culture to epidemiology. Presenters and Chairs who are from industry and academia, MDs and PhDs, junior and senior investigators, diverse in sex, race, and ethnicity, from across the US and Canada, and studying a variety of model systems will provide diverse perspectives on this issue. In summary, this Workshop will provide an overview of some of the sex-specific responses to respiratory toxicants and potential mechanisms elucidated from multiple models and a variety of respiratory toxicants.

W 2487 Influence of Sex and Sex Hormones on Lung Function and Disease: Research, Risk, and Regulatory Insights

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The roles of sex and sex hormones in lung function and disease are complex and not completely understood. In humans, sex hormones appear to exert regulatory effects on lung development before and during the neonatal period, with anatomical and functional differences between the sexes observed through to adulthood. Sex differences also exist in the prevalence of asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, and other lung conditions. Delineating the underlying contribution of sex (and, by association, sex hormones) to these diseases is complicated by the inherent difficulties associated with identifying the roles of specific factors from epidemiologic data. As a result, the potential contribution of sex hormones to sex disparities in numerous toxicities and diseases of the lung has been evaluated by various research groups using animal models under more controlled conditions, with results reported in the published scientific literature. In mice, sex differences have been reported in baseline lung function parameters and in the airway response to cholinergic stimulation. Moreover, other studies have documented sex differences and sex hormone-mediated influences in mouse models of lipopolysaccharide-induced inflammation, allergic lung inflammation, pulmonary fibrosis, tobacco smoke-induced injury, ozone-induced inflammation, and others. These investigations have confirmed the need to consider sex in the design and evaluation of animal models of lung toxicities and diseases. This is consistent with expectations for high-quality studies that are used for health risk evaluations, such as the derivation of inhalation occupational exposure limits, and with regulatory expectations for toxicology studies conducted to support drug development programs. Continued

study of the impact of sex and sex hormones will serve to strengthen the understanding of exposure risks and of potential strategies to address lung toxicities and diseases that may have a sex-specific component.

2488 Murine *In Utero* Secondhand Smoke Exposure Promotes Urethane-Induced Lung Tumors in Males and Metastases in Females via Extracellular Matrix-Remodeling

A. Noël, and A. Penn. *Louisiana State University, Baton Rouge, LA.*

Recently, it has been shown that lung cancer incidence is higher among young women than young men—a phenomenon that was not completely explained by differences in sex-specific smoking behaviors. Additionally, while secondhand smoke (SHS) exposure is a risk factor for lung cancer, it is unclear whether there is an association between *in utero* SHS exposure and lung cancer. We previously identified that *in utero* exposure to SHS caused, in adult mice, upregulation of miR-155-5p, miR-21-3p, and miR-18a-5p and downregulation of 16 tumor suppressor genes. This suggests that *in utero* SHS exposure may increase the susceptibility in adulthood to lung cancer development. This presentation will outline investigations of how *in utero* SHS exposure promotes the development of urethane-induced carcinomas, intrapulmonary metastases/emboli at 58 weeks of age, in both male and female mice. Pregnant Balb/c mice were exposed gestationally to 10 mg/m3 of SHS or filtered air. In adulthood, offspring were treated with intraperitoneal injections of urethane (1 g/kg) or saline. At 58 weeks of age, mice were sacrificed, and tumors were analyzed for number, volume, histopathological classification, and gene expression. SHS-exposed males showed increased numbers of tumors and tumor volume compared with control males. SHS males differentially expressed genes were part of functional clusters associated with non-small cell lung cancer, tumor suppressors, and ras signaling pathway. This suggests that the increased numbers of tumors in SHS males may be associated with increased risk of cancer progression. In females, SHS-exposed animals had an increased number of tumors, tumor volume, number of intrapulmonary metastases/emboli, and the percentage of lymphocytes in BALF, compared with female controls. In addition, SHS-exposed females showed dysregulated cancer-related gene expression associated with increased risk of metastasis. In this study, we demonstrate that *in utero* SHS exposures promote large tumors through dysregulation of tumor suppressor and ras signaling genes in male, while promoting metastasis through matrix remodeling in female mice. This is consistent with previous reports that demonstrated that estrogen plays a role in proliferation and can influence metastasis without affecting tumor growth.

2489 The Role of Gonadal Hormones in Sex-Specific Lung Inflammatory Response to Ozone

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Ozone exposure contributes to impairment of lung innate immunity. Emerging evidence suggests that lung diseases affect men and women disproportionately and that fluctuations of circulating hormone levels can affect inflammatory responses. Here, we hypothesized that 17ß-estradiol regulates inflammation and airway hyperresponsiveness (AHR) triggered by ozone exposure. We performed gonadectomy and hormone replacement (17ß- estradiol, 2 weeks) in C57BL/6J male and female mice, and exposed animals to 1 ppm of ozone or filtered air for 3 hours. We compared lung function parameters, expression of cytokines/chemokines in lung tissue, and lipocalin-2 levels in bronchoalveolar lavage fluid (BALF). We found significant changes in respiratory parameters and gene expression in males and females after ozone exposure. These were affected by gonadectomy and 17ß-estradiol treatment in a sex-specific manner. Gonadectomized male mice displayed higher AHR than controls (sham) exposed to ozone, and hormone treatment ameliorated this response. In females, ovariectomy reduced ozone-induced AHR, which was restored by 17ß-estradiol treatment. Ozone exposure increased lipocalin-2 levels in both females and males, and these were reduced in gonadectomized mice. Treatment with 17ß-estradiol restored lipocalin-2 in females but lowered it in males. Gonadectomy affected expression of inflammatory genes induced by ozone exposure in both males and females; expression of IL-6 and MIP-3 was strongly dependent on 17ß-estradiol levels in females. Together, these results indicate that sex hormones modulate ozone-induced inflammation and AHR. Future studies examining diseases associated with air pollution exposure should consider the patient's sex and hormonal status.

2490 Sex-Dependent Health Impacts of California Wildfire $PM_{2.5}$ across the Life Span

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Climate change has contributed to an increased incidence of wildfire events due to dry weather and overgrowth of vegetation. Prescribed burns and wildfires are now recognized by the US Environmental Protection Agency as significant sources of air pollution in the United States. Acute wildfire smoke exposure is associated with increased respiratory morbidity, including asthma symptoms and medication use, obstructive airways disease exacerbations, and hospitalization. Wildfire events are a growing national public health concern and have the potential for increased burden of disease; however, there is little known about the long-term health outcomes of acute wood/biomass smoke inhalation. To address this knowledge gap, we noninvasively and progressively monitored the health status of an outdoor colony of California National Primate Research Center rhesus monkeys over 10 years following exposure to ambient smoke from the Trinity and Humboldt County wildfires in 2008, with the rationale that nonhuman primates may serve as an animal surrogate for pediatric populations. At three years of age, adolescent rhesus monkeys that were exposed as infants displayed significant attenuation of responsiveness to microbial ligands in peripheral blood *ex vivo* in a sex-dependent manner, suggesting a persistent immune effect of air pollutants. Exposed adolescent animals also demonstrated significant alterations in lung function parameters, including reduced compliance in female monkeys. In a follow-up study at eight years of age, adult female monkeys continued to show evidence of immune dysregulation in peripheral blood after infant exposure. Using high-resolution computerized tomography to image the female adult monkey chest wall, we determined that early-life exposure to wildfire smoke significantly correlated with changes in lung volume, physiologic capacity, and lung remodeling in adulthood relative to unexposed age-matched counterparts. Ongoing studies following the recent Butte County wildfires in 2018 will monitor the health impacts of ambient smoke exposure on a cohort of pregnant and infant rhesus monkeys at the center. Collectively, our findings in a colony of nonhuman primates suggest that age of exposure and sex contribute toward vulnerability to long-term health outcomes following acute exposure to wildfire smoke.

2491 Disentangling Effects of *In Utero* Exposure to Ambient Air Pollutants, Prenatal Stress, and Fetal Sex on Respiratory Outcomes in Epidemiologic Studies

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Sponsor: M. Rebuli

The influence of outdoor air pollution on respiratory health starts *in utero*. Fetal lung growth and structural development occurs in stages; thus, effects on postnatal respiratory disorders may differ based on both exposure dose and timing of exposure. Moreover, the impact of prenatal ambient pollution exposure upon children's respiratory outcomes may be modified by infant sex as well as exposure to other toxins such as psychosocial stress. Our group has implemented innovative data-driven methods to identify sensitive windows for effects of prenatal exposure to ambient pollutants (fine particulate matter, nitrates, and their mixtures) on children's asthma development in an urban pregnancy cohort, which also allows for examining effect modification by infant sex and other exposures. These windows correspond to specific developmental processes that may be altered by environmental insults and likely vary depending on the mechanisms of the chemical exposures that may impact specific aspects of development. Methods that lead to a better understanding of how these windows of vulnerability differ by infant sex can lead to new insights into underlying mechanisms programming differential risk based on sex.

2492 Protecting Public Health and the Environment during Wildfire Recovery

S. DuTeaux. *California Department of Pesticide Regulation, Sacramento, CA.*

California is the only state that mandates cleanup activities following wildfires and assists impacted homeowners via a comprehensive remediation program. In most states, property owners deal with the destruction left in the wake of a wildfire largely on their own. These uncoordinated recovery activities have led to questionable and often hazardous ways of dealing with debris and ash, and can result in a legacy of contamination for affected communities. California emergency response and environmental protection

agencies joined forces in 2007 to develop a coordinated approach to remediating properties impacted by wildfires, which includes expedited removal of immediate hazards, asbestos abatement, and fire ash and debris removal. This unique and public health-protective approach supports communities in safely rebuilding. This session will outline the principles underpinning the California model; describe the steps involved in removing contaminants and clearing properties; present results from ash, air, soil, and water sampling in the aftermath of the fires; and discuss how public health, occupational health, and the environment are protected along the way. The presentations in this session will be given by State of California employees who were deployed under the Governor's Declaration of Emergency to work on wildfire recovery in Northern and Southern California following the November 2018 Camp and Woolsey wildfires and who will be drawing from their "boots on the ground" experience.

RI 2493 Overview: Why a Recovery Program after Wildfires with Urban Interface is Critical to Protecting Public Health and the Environment

M. Santillano. *California Environmental Protection Agency, Sacramento, CA.* Sponsor: S. DuTeaux

On November 8, 2018, multiple wildfires broke out in California. The Camp Fire was the deadliest and most destructive wildfire in California history to date. The fire started in the small community of Pulga in Butte County, in Northern California. Exhibiting extreme fire behavior and gale force winds, the Camp Fire ripped through the communities of Concow and parts of Magalia and virtually decimated the town of Paradise. In total, 19,000 structures on almost 14,000 properties were lost. The fire claimed the lives of 85 victims. After the fire was contained, an estimated 240 square miles of forested and populated areas were destroyed. The Camp Fire started around 6:30 am on November 8. A mere eight hours later, under the same dry conditions and severe winds, the Woolsey Fire broke out in Southern California. The fire started on the Santa Susanna Field Laboratory property, an industrial research and development complex operated by Boeing near the boundary of Los Angeles and Ventura counties. When it was contained, the Woolsey Fire left in its wake over 1,600 destroyed structures, three fatalities, and the evacuation of close to 300,000 people. How do communities recover from such devastation? This session will provide an overview of how California environmental and emergency management leaders developed a model for the safe cleanup of fire ash and debris, and how this model has been used to successfully recover from wildfires with urban interface in California since 2007. The session will go into detail about how the cleanup process is accomplished. It also will review the contaminants monitored and how they are detected in air, ash, soil, and water samples. The session presenters hope to show how California's consolidated recovery efforts can be a model for wildfire cleanup in other states.

RI 2494 Fire Ash and Debris Removal: Phases I and II of Wildfire Recovery

L. Chumney. *California Environmental Protection Agency, Sacramento, CA.* Sponsor: S. Iyer

When a typical home burns, all the contents can be destroyed, including major appliances, electronics, furniture, and cars. During this process, numerous hazardous substances can be released into the environment. These can include flame retardants, plasticizers, home and garden pesticides, lead and other metals, asbestos, and highly toxic combustion by-products. Cleaning up thousands of properties can be overwhelming for affected communities. The State of California's consolidated wildfire debris removal program paves the way for communities to start rebuilding after a devastating natural disaster by expediting the safe removal of fire ash and debris in a stepwise manner. The first and most important action that follows a California wildfire is the declaration of a local public health emergency. This establishes the government's authority to enter private property to abate immediately hazardous conditions without property owner permission. This action initiates Phase I of the wildfire recovery process by allowing hazardous materials teams to remove bulk hazardous materials, such as partially burned pesticide and paint containers, unexploded propane tanks, and unspent munitions. Once these immediate hazards are removed, the next phase of debris removal can commence. Phase II consists of a series of coordinated steps, including site assessment, asbestos abatement, metal and concrete segregation, ash and debris removal, and soil confirmation sampling and clearance. This second phase requires homeowner permission, since the immediate threats have been removed, and is usually coordinated between state agencies and the county environmental health department. This presentation will discuss the implementation of Phase I and II wildfire recovery efforts for the recent California wildfires. Specific sensitivities when working in impacted communities also will be discussed.

RI 2495 What Is in the Ash and How Do We Keep It from Getting in the Air?

S. DuTeaux. *California Department of Pesticide Regulation, Sacramento, CA.*

One of the biggest concerns for communities in the aftermath of a wildfire is exposure to the contaminants in ash. As repopulation begins, residents may find themselves surrounded by decimated properties, twisted and melted metal, and piles of ash. It is understandable that they would be concerned about what is in the ash and what they are breathing. Recovery efforts for California wildfires include strict dust mitigation efforts and community and site-specific air monitoring and sampling designed to detect if any ash from debris removal activities becomes airborne. Comprehensive ash sampling was conducted following major wildfires in California in 2003, 2007, and 2015. For wildfires with urban interface, the ash typically contains elevated concentrations of several metals, with arsenic, cadmium, copper, lead, nickel, and zinc usually found in the highest levels. Polycyclic aromatic hydrocarbons (PAHs) have been sampled following previous fires but were found mainly in concentrations below residential California Human Health Screening Levels (established by the California Environmental Protection Agency) and Preliminary Remediation Goals (established by the US Environemtnal Protection Agency). Comprehensive ash sampling is being conducted for the Camp Fire. With results pending, the screening levels for airborne contaminants were based on what was known from previous ash analysis. Air sampling was conducted for asbestos and the heavy metals listed in Title 22 of the California Code of Regulations, Section 66261.24 ["Environmental Health Standards for the Management of Hazardous Waste"]. Air quality also was monitored for particulate matter (both $PM_{2.5}$ and PM_{10}) using the National Ambient Air Quality Standards and the values in the Wildfire Smoke Guide as screening levels. This presentation will cover the latest ash characterization data and the major contaminants of concern found in the Paradise area. Details will be shared on how sampling times, durations, locations, and screening levels were chosen for the different air monitoring and sampling approaches, as well as data on the airborne excursions (metals, asbestos, and PM), and if those detections were associated with debris removal activities. This presentation also will cover special air quality activities required around repopulated areas and public outreach on air quality data.

RI 2496 Fire Ash and Debris Removal: Quantitation of Success via Confirmation Soil Sampling

S. Iyer. *California Environmental Protection Agency, Oakland, CA.*

California's consolidated debris removal program for areas impacted by wildfires uses soil testing results to evaluate site cleanup. The approach of comparing soil contaminant levels to health-based or other standards is integral to assessing sites with hazardous materials spills, decommissioned military bases, and Superfund sites to determine if they are safe enough for rebuilding or repurposing. California state environmental officials created and implemented a methodology tailored for the unique contamination that can result from wildfires with urban interface, based on the characterization of hazardous constituents found in burn debris and ash from previous fires. Soil samples collected following debris removal from an impacted property are tested for the metals specified under Title 22, California Code of Regulations, Section 66261.24. These include antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, silver, thallium, vanadium, and zinc. The soil samples are analyzed with US Environmental Protection Agency Method 6020. The number of soil samples collected per cleared parcel depends on the size of the original ash footprint; typically, at least one sample is collected for every 100 square feet. California develops project-specific cleanup goals for each metal, based on state or federal health-based standards, or background soil concentrations. Background soil samples are collected from the vicinity of the wildfire, excluding the ash-impacted areas, and are used to establish local metal concentrations. Establishing these background levels is critical for meeting the debris removal program's goal of removing ash and debris related to the recent wildfire incident. If initial soil testing results from cleared parcels fail to meet the project cleanup goals, additional soil is scraped from the parcel, and confirmation soil testing is repeated. Once the soil samples meet the cleanup goals, erosion control measures are installed, and the property is ready for rebuilding. This presentation will explain the soil sampling protocol and will review available soil data from recent California wildfires.

2497 Water, Water Everywhere: Efforts to Protect Drinking Water and Sensitive Aquatic Species

B. Stanton. *California Environmental Protection Agency, Sacramento, CA.* Sponsor: S. Iyer

Years ago, wildfires across the western United States generally occurred during the hot and dry months of summer and early fall. The reality for western states is that there is no longer a "wildfire season." Wildfires have ignited in California in November, December, and even January. The Camp wildfire occurred in November 2018 and was fully contained just prior to the start of a record rainy season, making it very difficult to clear debris because of the wet conditions. This resulted in consecutive months where fire ash and debris sat untouched. The inevitable question was whether any contamination migrated off-site from thousands of destroyed properties, and if there were any impacts to drinking water and aquatic life. Erosion control efforts are critical in many areas in California following wildfires because so much of the population receives its drinking water from surface water sources and because of impacts to critical species, such as winter run Chinook salmon. In the Paradise area, results from surface water analysis conducted from January to April 2019 showed levels of certain contaminants exceeding water quality standards for drinking water in areas impacted by the Camp Fire. Preliminary laboratory analyses found concentrations of aluminum, antimony, arsenic, cadmium, lead, and selenium exceeding Primary Maximum Contaminant Levels (MCLs) at most monitoring stations. PAH concentrations also were elevated but did not exceed the MCL. Such contamination could potentially impact repopulated areas that use untreated surface water or private wells for drinking water. Contaminants in fire ash also could potentially impact aquatic species if ash enters the watershed. This presentation will cover the efforts to protect water from contamination following wildfires with urban interface, additional considerations for seasonal waterways, and the measured impact to surface water from the fires.

2498 Using the Key Characteristics of Carcinogens to Organize Mechanistic Data for Acetaminophen

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Acetaminophen (paracetamol) is one of the most widely used medications for pain relief and fever reduction. Many new studies have been published since 1999, when it was evaluated by IARC and classified as a Group 3 carcinogen. We recently evaluated the evidence of carcinogenicity for acetaminophen by reviewing data from relevant human, animal, and mechanistic studies. Here we present our approach to the evaluation of mechanistic data, which identified and organized data according to the 10 key characteristics (KCs) of carcinogens (Smith et al., EHP 124:713, 2016, IARC 2019). As such, our review included data on metabolism, genotoxicity, DNA repair, epigenetic alterations, oxidative stress, immune and inflammatory responses, receptor-mediated effects and cell proliferation. Our review and analysis found that the available mechanistic data on acetaminophen provide support for four KCs, specifically, KC 1, 2, 3, and 5. Regarding KC 1, acetaminophen can be metabolized to form electrophilic compounds such as *N*-acetyl-*p*-benzoquinone imine and *p*-benzoquinone, and free radicals such as the *p*-aminophenoxy radical. Regarding KC 2, there is evidence that acetaminophen and several of its metabolites are genotoxic, e.g., inducing chromosomal and DNA damage, including at doses that fall within the therapeutic range. Regarding KC 3, acetaminophen and/or its metabolites have been observed to alter DNA repair via inhibition of ribonucleotide reductase and decreased 8-oxoguanine DNA glycosylase activity, and are associated with genomic instability as measured by increases in γ-H2AX, a marker of double-strand breaks, and by inhibiting DNA topoisomerase IIα. Regarding KC 5, there is evidence that acetaminophen induces oxidative stress, based on data from *in vivo* and *in vitro* studies conducted in humans and animals, even at sub-toxic doses. Results of omics studies also indicate the induction of oxidative stress. This work demonstrates how the KCs of carcinogens can facilitate the organization of complex mechanistic data that are pertinent to carcinogenesis, and thus provide insights on the potential mechanisms of action for acetaminophen.

2499 Loss of SWI/SNF Chromatin Remodeling Activates NRF2 Signaling in Non-small Cell Lung Carcinoma

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The NF-E2-related factor 2 (NFE2L2) transcription factor, also known as NRF2, induces expression of cytoprotective genes upon cellular exposure to oxidative stress. Next-generation sequencing studies of lung cancer have shown a significant number of activating mutations within the NRF2 signaling pathway. Mutations in components of the SWI/SNF chromatin-remodeling complex, a general regulator of transcription employing either SMARCA4/BRG1 or SMARCA2/BRM as the catalytic subunit, also frequently occur in lung cancers. Here, we show that loss of SWI/SNF complex function activated a subset of NRF2-mediated transcriptional targets. Using a series of isogenic NSCLC lines with reduced or depleted BRG1 and/or BRM expression, we observed significantly increased expression and protein levels of the NRF2-target genes HMOX1 and GSTM4. Expression of the NRF2 target genes NQO1 and GCLM modestly increased following BRM reduction but were not affected by BRG1 suppression. High-throughput RNA sequencing of BRG1-proficient and -deficient cell lines confirmed these changes in KEAP1-NRF2 target gene expression as well as identified novel targets of the SWI/SNF complex. Importantly, low BRG1 expression levels in primary human NSCLC correlated with increased NRF2-target gene expression. Our data demonstrate that loss of BRG1 or BRM in lung cancer results in activation of the NRF2/KEAP1 pathway and increased expression of a subset of its downstream targets. Therefore, we provide an additional molecular explanation for why patients harboring BRG1 or BRM mutations show a poor prognosis. Our results also potentially link mutations in SWI/SNF complex components, that occur in >20% of human cancers, with exposure to environmental toxicants. Additionally, a better understanding of these mechanism may yield novel insights into the design of targeted treatment modalities for human lung cancers.

2500 Use of the 10 Key Characteristics of Carcinogens and Evidence Integration in National Toxicology Program (NTP) Cancer Hazard Assessments

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NTP conducts literature-based cancer hazard evaluations, usually to review substances for listing in the Report on Carcinogens (RoC). Following guidelines in the RoC Handbook, these assessments use systematic review methods and integrate evidence from human, experimental animal, and mechanistic studies. Recognizing the increasing importance of mechanistic data in hazard assessments and the challenges in identifying informative studies from a large and diverse literature base, the NTP has adopted a structured approach using the 10 key characteristics of carcinogens (KCs) to search, organize, and review mechanistic information. Here we describe the use of the KCs in several NTP cancer hazard assessments: antimony trioxide, haloacetic acids (HAAs), night shift work (NSW), and light at night (LAN). In each assessment, we searched PubMed, Scopus, and Web of Science using terms targeting each KC. Mechanistic data evidence maps were developed by screening and tagging the literature by KCs and other relevant data (e.g., toxicokinetics and biological effects other than KCs) using an online content-management system. For NSW and LAN, studies of circadian disruption were also tagged by KCs. The next steps were to evaluate the studies, synthesize the evidence across KCs, and integrate the evidence with other data relevant to cancer. For each assessment, the evidence indicated that exposure to the substance (or exposure scenario) was associated with several specific KCs. For some KCs, there were too few studies to assess the strength of the evidence, thus, evidence mapping by KCs may help to identify data gaps. For assessments that did not have human cancer data (antimony and HAAs), the KCs informed biological plausibility. In the HAA assessment, consistent trends in toxicokinetics and KCs were related to the halogen substitution patterns across the 13 HAAs. These trends were used in read-across approaches that supported listing 2 HAAs that did not have animal cancer data. A primary strength of the NSW and LAN evaluations was that the database included some cancer studies that also evaluated KCs or other mechanistic effects and provided evidence of a direct contribution to cancer. In all recent NTP cancer hazard assessments, the 10 KCs provided an effective framework for identifying, organizing, and integrating mechanistic data with human and animal cancer data and other relevant information.

2501 Role of Endothelial RhoA-ROCK Pathway in Cancer Metastasis

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Metastasis, the cause of over 90% of cancer-related deaths, is the principal complication during cancer therapy. An important step in the metastatic process is the migration of the cancer cells through the endothelial lining of vasculature during intravasation and extravasation. The molecular mechanisms underlying trans-endothelial migration, is poorly understood. It has been shown that endothelial RhoA-Rho kinase (ROCK) pathway is involved in vascular permeability. Here, we investigated the role of endothelial RhoA-ROCK pathway *in trans*-endothelial migration and cancer metastasis. *In vitro*, we used a quantifiable, highly reproducible, transwell-based, two-cell co-culture model of trans-endothelial migration, where fluorescently-labeled cancer cells transmigrate through an endothelial layer. Both human primary and immortalized endothelial cells, were used and a wide range of human and murine cancer cells were tested. Endothelial RhoA-ROCK pathway was blocked by pharmacological inhibitors or siRNA. The number of cancer cells migrating through the endothelial monolayer was quantified and compared to the controls. To measure endothelial RhoA activation, RhoA pull-down assay was performed with cancer cell supernatant. *In vivo*, endothelial-specific *RhoA* knockout mice were used in experimental metastasis models, where syngeneic murine cancer cell lines B16-F10, LLC-LL3 and E0771 were administered, using both intravenous and intracardiac model. The number of metastatic lung nodules was quantified from the excised lungs. Fasudil, a clinically relevant inhibitor of the RhoA-ROCK pathway was used. A variety of different cancer cell lines of both murine and human origin were able to potently activate endothelial RhoA. *In vitro*, treatment of endothelial cells with either Rho inhibitor, C3 toxin or siRNA for RhoA decreased the trans-endothelial migration of all the cancer cells tested. Similar results were observed when Rho kinase (ROCK) was inhibited using Fasudil or Y-27632. *In vivo*, we observed decreased number of metastatic foci in endothelial-specific RhoA-deficient mice compared to the littermate controls for all the cancer cells tested. Treatment with Fasudil, significantly decreased the metastatic colonization of both human and murine cancer cells. Collectively, our findings highlight the role of endothelial RhoA-ROCK signaling in cancer cell trans-endothelial migration and metastasis. Blockade of this pathway showed a better outcome in cancer metastasis, highlighting the potential of Fasudil as an anti-metastatic drug candidate.

2502 High-Fat Diet Unmasks Tumorigenic Potential of Transient Vinyl Chloride Exposure at Sub-OSHA-Level Concentrations

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Vinyl chloride (VC) monomer is volatile organic compound used in the industry to produce polyvinyl chloride (PVC). At high exposure levels VC causes hepatic angiosarcoma and toxicant associated steatohepatitis. However, we have recently shown that lower exposure levels (i.e., < OSHA exposure limits), which do not directly damage the liver, enhance injury caused by high fat diet (HFD). Although these lower exposure levels are currently considered 'safe', it is unknown if the long-term impact of transient low-concentration VC enhances the risk for the development of liver cancer. This is especially a concern given the fact that fatty liver disease is in and of itself a risk factor for the development of liver cancer. To evaluate the long-term effect of VC exposure C57Bl/6J mice were fed HFD, or low-fat control diet (LFD) for 1 year. During the first 12 weeks of feeding, mice were also exposed to VC in inhalation chambers at concentrations below the current OSHA limit (<1 ppm) or room air for 6 hours/day, 5 days/week. The remaining 9 months of feeding, mice were not exposed to VC. Plasma and liver samples were collected for histology and determination of liver damage. As expected, chronic LFD feeding did not cause hepatic injury in the presence or absence of VC. VC did, however, significantly enhance hepatic steatosis in the LFD group. Feeding HFD for 1 year caused significant hepatic injury, including steatohepatitis and moderate fibrosis, which was exacerbated by VC. Specifically, HFD increased indices of oxidative stress (4-HNE) and of fibrosis (Sirius Red, fibrin) which were significantly increased by VC exposure. Additionally, HFD feeding induced the formation of some, mostly benign tumors. In the HFD+VC group the number of tumors was significantly increased. Tumors in the HFD+VC group ranged from moderately to poorly differentiated HCC with clear basophilic cytoplasm, moderate to high nuclear atypia and tumor cells were without steatosis. Importantly, mice in this group also had an increase in CD31, and potential endothelium-derived tumors, which may be resembling VC-induced hemangiosarcoma seen in humans. Our data indicate that VC sensitizes the liver to other stressors (e.g., HFD) resulting in enhanced tumorigenesis. These data raise concerns about potential interaction between VC exposure and HFD diet (Western diet). Further it also emphasizes that current OSHA safety restriction may be insufficient to account for other factors that can influence hepatotoxicity.

2503 Novel Mechanism of Liver Carcinogenesis Mediated by Peroxisome Proliferator-Activated Receptor-α (PPARα)

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Chronic administration of PPARα agonists causes hepatocarcinogenesis in rodents through a mechanism mediated by PPARα. A large body of evidence indicates that the hepatocarcinogenic effect of PPARα agonists is rodent-specific and humans are refractory to this. Administration of the potent human PPARα agonist GW7647 for up to 18 months caused nearly 100% incidence of hepatocarcinogenesis in wild-type mice. This effect was diminished in *Ppara*-null and humanized *PPARα* (h*PPARα*) mice but a low incidence of hepatocarcinogenesis was observed in these mice, independent of treatment. The mechanism by which *Ppara*-null and h*PPARα* mice spontaneously develop liver cancer was examined in the present study. Metabolomic analyses revealed that hepatic fatty acids increased in *Ppara*-null and h*PPARα* mice, independent of GW7647 treatment and this effect was not observed in wild-type mice. Moreover, *Ppara*-null and h*PPARα* mice had higher hepatic levels of unsaturated fatty acids including polyunsaturated fatty acids (PUFA). GC-MS detected an increase in PUFAs including linoleic acid in the liver of *Ppara*-null and h*PPARα* mice, independent of ligand activation of PPARα by GW7647. Since linoleic acid can selectively cause $CD4^+$ T lymphocytes loss and promote hepatocarcinogenesis, it is of interest to note that $CD4^+$ T lymphocytes were decreased in *Ppara*-null and h*PPARα* mice compared to wild-type mice. Additionally, serum IFN-γ and TNF-α, which are essential for $CD4^+$ T cell-dependent immune surveillance, were significantly decreased in *Ppara*-null and h*PPARα* mice compared to the control. Hepatic 8-oxo-dG DNA adducts exhibited an age-dependent increase in all genotypes. These studies suggest that genetic silencing of mouse PPARα causes increased hepatic linoleic acid in *Ppara*-null and h*PPARα* mice. This change is associated with reduced hepatic level of $CD4^+$ T cells that is associated with decreased levels of serum IFN-γ and TNF-α, two biomarkers of $CD4^+$ T cell activity. Combined, this suggests that liver tumors found in *Ppara*-null and h*PPARα* mice are due to reduced immune surveillance leading to age-related hepatic cancer. Since there are no known null mutations for PPARα in nature, and this mechanism is not related to ligand activation of PPARα, it must be considered when assessing the phenotype of *Ppara*-null and h*PPARα* mice.

2504 Role of Novel HDAC Inhibitors in Castration Resistant Prostate Cancers

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Castration resistant prostate cancer is the second most common cause of death from cancers in men. Overexpression of histone deacetylases (HDACs) 1 and 2 is linked to poor prognosis in prostate cancer, making HDACs an enticing target. However, clinical trials using HDAC inhibitors have failed owing to toxicity in patients, poor pharmacokinetic profile and evolution of resistance. Therefore, *N*1-hydroxy-*N*^{8}-(4-(pyridine-2-carbothioamido)phenyl)octanediamide 1 and [chlorido(η^5-pentamethylcyclopentadienyl)(*N*1-hydroxy-*N*^{8}-(4-(pyridine-2-carbothioamido-κ^2*N,S*)phenyl)octanediamide)rhodium(III)] chloride 2 were synthesized and tested in comparison with the clinically used HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA). HDAC inhibition, acetylation induction, cytotoxicity and anti-angiogenic potential were assessed in androgen receptor (AR)-positive LnCAP, AR-negative Du145 and PC3 prostate cancer cell lines and normal prostate cells PNT1A6. HDAC inhibition assays revealed that equipotent inhibition was observed for SAHA, 1 and 2. Docking studies using 4LXZ (SAHA co-crystallized with HDAC2) revealed that 1 coordinates via sulfur and nitrogen with the zinc ion, whereas one pose also revealed that it coordinates to the zinc ion via the hydroxamate group. Acetylation of histone variant-3 was also induced in response to 1 and 2 in PC3, Du145 and PNT1A6 cells. As determined by sulforhodamine B assays, 1 and 2 had EC_{50} values of 2.37 and 3.06 μM, 1.81 and 7.21 μM, 5.41 and 9.38μM, 2.81 and 3.24μM in PC3, Du145, PNT1A6 and LnCAP cells respectively. Of note, 1 and 2 were less cytotoxic towards PNT1A6 with EC_{50} values of 5.41 and 9.38 μM respectively than SAHA (EC_{50}= 1.96 μM). Anti-angiogenic roles were explored using Western blotting of VEGFR-2 and VEGF-A in PC3 cells; 1 and 2 reduced their expression, in contrast, SAHA increased the levels of VEGFR2. Tube formation assays using HUVEC cells revealed a significant reduction of

24.14% and 27.4% of total segment length per field in response to 1 and 2 as compared to control. Both 1 and 2 inhibit HDACs, induce acetylation, possess anti-angiogenic activity and are cytotoxic against prostate cancer cells. Thus, examination of pharmacokinetic and toxicity profile of these drugs in *in vivo* models is warranted.

2505 Chronic Arsenic Exposure Increases Metastatic Potential of Lung Cancer Cells via Transcriptional Regulation of SOX9 by NRF2

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Chronic arsenic exposure continues to be a worldwide health concern because of its prevalence and contribution to disease, specifically tumorigenesis and, as recently evidenced, metastasis. Non-small cell lung cancer (NSCLC) patients that develop a metastatic lesion have a 5-year survival rate of 6%, as compared to 60% in localized tumors; however, the exact mechanism for what causes NSCLC cells to metastasize is unknown. One proposed contributor is nuclear factor (erythroid-derived 2)-like 2 (NRF2), a transcription factor with known oncogenic properties that is induced by arsenic toxicity. Under basal conditions, a Ring-Box 1-Cullin-3 (RBX1-CUL3) E3 ubiquitin ligase complex maintains low NRF2 expression via constant ubiquitylation and degradation; however, alterations to Kelch-like ECH-associated protein 1 (KEAP1), a substrate adaptor protein of the RBX1-CUL3 complex that binds NRF2, either by cysteine oxidation, mutation, or p62-dependent sequestration, allows NRF2 to translocate into the nucleus, bind to antioxidant response elements (ARE), and promote transcription of its target genes. Here, we demonstrate that chronic arsenic exposure causes malignant transformation of normal immortalized lung epithelial cells (BEAS-2B) and aids in invasion and migration via NRF2-dependent upregulation of SRY-box 9 (SOX9): a transcription factor linked with metastasis. Upon analysis of the *Sox9* gene, we confirmed the presence of a functional ARE sequence in its promoter region, suggesting that NRF2 is responsible for controlling SOX9 expression. To verify, we demonstrated that pharmacological induction or inhibition of NRF2 increased or decreased SOX9 expression, respectively. Additionally, we showed that mutation of the ARE sequence upstream of the *Sox9* gene prevented NRF2 binding, thus blocking transcription of SOX9. As increased proliferation is linked with tumorigenesis, we utilized NSCLC cells (H1299) to demonstrate that heightened activation of NRF2 via knockout of KEAP1 contributes to proliferation; while, inhibition of NRF2 or direct knockdown of SOX9 reduced proliferation in both a wild type and constitutively active NRF2 setting. To further elucidate the role of NRF2 in metastasis, we also showed that loss of NRF2 or SOX9 slowed the ability of cancer cells to migrate and invade. Overall, this evidence suggests that NRF2 control of SOX9 expression can contribute to the metastatic potential of both environmentally and genetically driven lung tumors.

2506 Role of AhR Ligands in miR-132-Mediated Modulation of HMBG1 in Inducing Th17/T Regulatory Cell Differentiation in Delayed Type Hypersensitivity

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The aryl hydrocarbon receptor (AhR) is known to have an impact on immunomodulation. Recent data has shown that TCDD, an exogenous AhR ligand, induces T regulatory cells (Tregs), while FICZ, an endogenous AhR ligand, induces Th17 cells. miR-132 has been involved in autoimmune encephalomyelitis. The aim of this present study is to investigate the effects of TCDD and FICZ on T cell differentiation in delayed type hypersensitivity (DTH) by using miR-132 KO mice. Treatment of C57BL/6 mice with TCDD attenuated DTH responses to methylated bovine serum albumin and induced Tregs. In contrast, treating DTH mice with FICZ induced pro-inflammatory Th17 cells. Analysis of microRNA (miR) profiles from draining lymph nodes showed differential regulation between TCDD and FICZ groups. Specifically, miR-132, which was overexpressed in TCDD group, led to downregulation of the gene target, high mobility group box 1 (HMGB1). Downregulation of these gene targets led to an increase in Treg differentiation. In contrast, FICZ treatment caused a downregulation of miR-132, which leads to an upregulation of HMGB1. Treatment of miR-132 KO mice with TCDD or FICZ in DTH to validate this pathway showed that TCDD failed to suppress the immune response by increase in the footpad thickness and increase in the absolute number of cells in the draining LN. Furthermore, Foxp3 was significantly reduced due to increase in the HMGB1. On the other hand, FICZ treatment of miR-132 KO mice showed same results as the wildtype mice by increasing Th17 cells. In summary, miR-132 KO mice showed that TCDD and FICZ have divergent effects on miR-132 in a DTH model, and both ligands differentially regulate miR-132, which targets HMGB1 affecting key components involved in Th17 and Treg development. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.*

2507 Physiologic Expression of AhR Regulates Resident Tissue Macrophages in the Small Intestine and Alters Susceptibility to Type 1 Diabetes

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Susceptibility to type 1 diabetes (T1D) is influenced by both genetic and environmental factors. Identification of these environmental contributors has become increasingly urgent as a result of the steady rise in young children diagnosed with T1D over the past twenty years. Two environmental factors that have been associated with altered T1D risk are the diet and alterations in the intestinal microbiome. Metabolites derived from the diet and microbiome have been shown to activate the aryl hydrocarbon receptor (AhR), a ligand activated transcription factor that regulates the immune response. We therefore hypothesized that diet- and microbial- derived metabolites may influence the autoimmune response and subsequent susceptibility to type 1 diabetes. To test this hypothesis, we generated AhR knockout mice on the nonobese diabetic (NOD) background and examined the intestinal immune response and development of type 1 diabetes. Female AhR knockout mice had had reduced insulits at 12 weeks of age, and a decreased incidence and onset of overt diabetes in comparison to their wild type littermates. A similar trend was observed in AhR knockout and wildtype male mice. To determine whether physiologic expression of AhR was altering the immune response, we analyzed CD4+ T cell subsets in Peyer's patches, and the lamina propria and intraepithelial layer of the small intestine. While there were no significant changes in the percentage of Th17 cells at any tissue site, there was an increase in IL10R+Foxp3+ Tregs in the LPLs of AhR knockout mice. While analyzing intestinal CD4+ T cell populations, we also discovered a new population of resident-like macrophages in the small intestine lamina propria. These cells are negatively regulated by AhR, have a very strong positive correlation to lamina propria Tregs, and correlate with protection against insulitis in NOD mice. To identify the potential source of AhR ligands that regulate these resident-like macrophages, mice were fed an AhR ligand-deficient diet. These cells were not further regulated by dietary ligands, and therefore, it is likely that the AhR ligands originates from the intestinal microbiome. Future studies are planned to determine how AhR regulates the expression of lamina propria resident macrophages and to uncover their immunoregulatory function duringT1D pathogenesis.

2508 Validated Immunophenotyping Panels for the Assessment of Immunotoxicity in Nonhuman Primate, Humanized Mouse, Canine, and Rat Toxicology Studies

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Understanding how cells respond to drugs and other external stimuli is an important part of the drug development process. Because of the clinical success of the immune checkpoint inhibitors, the number of immune-modulating drugs in development has increased dramatically in the past 10 years. This includes drugs that are designed intentionally to be immune-activating, or immunosuppressive. In addition to expected effects, drugs can have unexpected and off-target toxicities on the immune and hematologic systems. Thus, monitoring immune cells is an important part of hazard identification and risk assessment in any well designed Toxicology study. Immunophenotyping is used to identify immune cell subsets based on antigens expressed on the cell surface, and can also include cytoplasmic and nuclear antigens. Specific antigens are identified using antibodies tagged with fluorescent dyes. Antibodies are used in combination, called a panel, to phenotype cells. Immune cells are most commonly analyzed from peripheral blood, but can also be analyzed from single cell suspensions prepared from the lymphoid organs (bone marrow, spleen, lymph node) using flow cytometry. Immunophenotyping relies heavily upon CD, or cluster of differentiation, markers on the cell surface. There are currently almost 400 CD markers, overseen by the International Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDAs). Non-clinical species commonly used for immunotoxicology studies include the non-human primate (NHP) for protein therapeutics, and rat and canine for small molecules. Cross-reactivity of antibodies to the CD markers of non-clinical species must be known in order to design immunophenotyping panels. Typically, rodent and canine antibodies are made to species-specific antigens, so cross-reactivity is not an issue. However, NHP antibodies are made to human CD antigens, so their cross-reactivity must be confirmed

(www.nhpreagents.org). Humanized mice are an invaluable tool for studying the human immune system, *in vivo* (and there are no issues with the cross reactivity of antibodies), and are increasingly used as an alternative to the NHP in toxicology testing. FCSL has developed and validated flow cytometry Immunophenotyping panels for monitoring the immune cells of four non-clinical species: NHP, canine, rat and humanized mice, for use on GLP and non-GLP studies.

2509 Differential Effects of the AhR on Immunoglobulin Expression in a Human B Cell Line

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Inhibition of antibody expression and secretion by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is well established in animal models. This inhibition is mediated by the aryl hydrocarbon receptor (AhR). However, the effect of TCDD on human B cells is less clear. Therefore, the objective of this study was to evaluate the effects of TCDD on Ig expression, via ELISA analysis and real-time PCR, in a human B-cell line (CL-01). CL-01 cells were originally generated from a Burkitt lymphoma patient. These cells can be stimulated by CD40L and IL-4 to secrete Ig and undergo class switch recombination. TCDD significantly inhibited IgG secretion but had no effect on IgM secretion. TCDD also altered Ig heavy chain (*IGH*) transcription, producing a significant decrease in γ1-4 transcripts and an increase in α1 and α2 transcripts. Co-treatment with an AhR antagonist not only reversed the effects of TCDD but also enhanced stimulation-induced γ1-4 expression and IgG secretion, perhaps due to the presence of endogenous AhR ligands in the media. These results support a role of the AhR in regulating human *IGH* expression.

2510 *In Vitro* Assay System to Assess Alterations of Thymocyte Development by Environmental Toxicant Exposures

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Endocrine disrupting chemicals (EDCs) are widespread and are in many common household items. These are chemicals that interfere with the body's endocrine system and can cause developmental, reproductive, neurological, and immunological effects by mimicking and/or interfering with the activity of hormones. Exposure to these chemicals may cause autoimmune diseases and have long-lasting effects. Previous research has shown that exposure to EDCs, such as Diethylstilbestrol (DES) and 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE; the primary metabolite of the pesticide methoxychlor), decreases cell viability and alters development of gestational day 16-18 C57BL/6 primary embryonic thymocytes during *in vitro* differentiation (Leung-Gurung et al., 2018). Effects were seen in the double positive population of developing thymocytes, the population of immature thymocytes that differentiates into mature CD4+ helper T-cells or mature CD8+ killer T-cells. Investigating effects on embryonic thymocyte differentiation in primary cells is cost- and labor-intensive. Until now, no cell line had been identified that could be used as a suitable replacement for primary cells in the differentiation assay to investigate the impacts of environmental toxicants on thymocyte development. In this study we explored using a double positive thymic lymphoma cell line, VL3-3M2. VL3-3M2 cells were cultured with and without CD2 and TCR antibodies (used to signal the cells to differentiate) and treated with 50pM, 50nM, and 50uM of DES or HPTE for 24 hours. The results from the VL3-3M2 cell line show similar effects as seen in primary embryonic thymocytes, including changes in cell viability and cell surface markers. This work establishes a new assay system for exploration of the alteration of thymocyte development by environmental toxicant exposure.

2511 Constructing and Challenging a Gastrointestinal Co-culture Model for Immunotoxicity

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In the field of nanotoxicology there is increasing interest in determining the immunological responses in the gut after ingestion of advanced materials used in the food and drug industry. To probe for oral exposure toxicities, a cell-based reproducible model for the gut is severely needed. To create a more comprehensive gastrointestinal *in vitro* model, three human cancerous cell types were co-cultured: HT29-MTX (intestinal mucous-producing goblet cells), Caco-2 (colon epithelial cells), and Raji B (lymphocytes). When Raji B and Caco-2 cells were co-cultured, a subpopulation of the Caco-2 epithelial cells transformed into endocytic, immune-supporting Microfold cells ("M cells"). The intestinal epithelial barrier model produced by this co-culturing method contained a "true" epithelial cell (Caco-2), a goblet cell (HT29-MTX), and two types of immunological cells (Raji B and differentiated M cells). This culture method allows for more robust immunotoxicological analyses. Furthermore, the combination of M cells and HT29-MTX cells is known to play an important role in endocytosis-mediated transepithelial absorption of particle like materials, thus allowing for a more comprehensive analysis of the naturally-polarized intestinal barrier nanomaterial uptake. Cellular communication and metabolic activity experiments, including cell viability, gastrointestinal barrier integrity, oxidative stress, and proinflammatory response markers, were used to characterize the model. After completion of baseline characterization, simulated cellulose digestate and undigested cellulose were used to challenge the model, and the model was also exposed to a positive control to induce toxicity. In comparison to monocultures of the three cell lines, the triculture has increased sensitivity to exposure and thus allows for a more realistic challenge-response analysis. This co-culture model and model challenge provides a foundation for reproducible future oral exposure toxicity analyses without the need for animal models.

2512 Historical Immunophenotyping Data in Preclinical Species

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Flow cytometry allows multiparametric analysis of thousands of particles per second and helps to adequately identify or functionally characterize complex cell populations of interest. It is a tool often used in basic research, discovery, preclinical and clinical trials. During the preclinical phase for the development of new drugs, flow cytometry has routinely been used for assessing the immunotoxic effects of a candidate drug by evaluating the immunophenotype of various cell populations in whole blood, lymphoid tissues, or other matrices. Despite the fact that immunophenotyping data is being generated by multiple different laboratories, combined database presentation and comparison of preclinical immunophenotyping data in various animals, strains and tissues/whole blood has been limited. The objective of this study was to compile relevant preclinical immunophenotyping data from monkeys (cynomolgus, rhesus), dogs (beagle), rats (Sprague-Dawley, Wistar, Fisher) and mice (various strains), from different matrices. The database from a single site was extracted from control data generated from over fifteen years of work. The specie, strain, age, cell population and sample type were grouped to provide a representation of expected lymphocyte population distribution profiles.

2513 Checkpoint Inhibitors Induce Immune-Mediated Liver Injury in Mice

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Inhibition of immune checkpoints (e.g. cytotoxic T-lymphocyte-associated protein 4 (CTLA-4); programmed cell death 1 (PD-1) and indoleamine 2,3-dioxygenase 1 (IDO1)) has demonstrated antitumor efficacy, especially in various combinations, in preclinical models and humans across several types of cancers, despite of immune-related adverse events. We developed a novel mouse model to elucidate the immune mechanisms of one of the more commonly seen adverse events, hepatitis, in the setting of combinatorial checkpoint blockade. PD-1-/- mice (C57BL/6) were treated with anti-CTLA4 (clone 9D9 mouse IgG2b, 300 µg/dose) intravenously weekly. Epacadostat (600 mg/kg) or vehicle control was administered twice a day by oral gavage. Each treatment group has 4 mice. Necropsy was performed after 2 weeks. CD45+ enriched liver cells (Miltenyi) were processed with the 10X genomics platform for single cell RNA sequencing. T cell receptor (TCR) and B cell receptor (BCR) repertoire profiling was performed in conjunction with gene expression. Single cell transcriptomics revealed that (1) the liver injury due to immune checkpoint inhibitors (ICIs) is associated with a population of CD8 T cells that express activation and exhaustion markers; and (2) ICI combination treatment decreased T cell clonality, indicating a broadening of the immune repertoire. This model could be of significant value to the greater scientific community in that it can provide mechanistic insight into immune-mediated liver toxicity associated with various drug combinations. It may provide circular guidance and support for clinical anti-cancer drug combination regimens that contain ICIs.

2514 Cannabinoid Receptor 2 Activation Exerts Anti-Inflammatory Effects on Primary Human CD8+ T Cells

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Combined antiretroviral therapy (cART) has altered the prognosis of HIV from a fatally progressive disease to a manageable chronic disease. However, an increase in neural inflammation is found to be associated with aging HIV infected individuals. The inflammation contributes to the etiology of HIV associated neurocognitive disorder (HAND) of which there are specific diagnoses with varying severity. While the underlying mechanism of HAND is still largely unknown, immune cells such as $CD8^+$ T cells have been observed to cross the blood brain barrier and produce pro-inflammatory cytokines, which may induce neuronal cell injury. Tetrahydrocannabidiol (THC) has been previously shown by our laboratory to reduce immune cell activation and production of pro-inflammatory cytokines. Therefore, due to the preferential expression of cannabinoid receptor (CB) 2 on lymphocytes, we hypothesize that activation of CB 2 by THC could reduce the activation and pro-inflammatory responses by human $CD8^+$ T cells, which will be confirmed by treatment with JWH-015, a selective CB2 agonist. Therefore, in the current studies, naïve human peripheral blood $CD8^+$ T cells from healthy subjects were activated by anti-CD3/anti-CD28 and treated with THC or JWH-015 followed by restimulation with PMA/Io after a six day incubation. A decrease in $CD8^+$ T cell activation marker expression, CD25 and CD107a, as well as inhibition of pro-inflammatory cytokine production, IFN-γ, were observed in cells treated with THC. *Supported by NIH R01-DA047180.*

2515 Strain Comparison of Sprague Dawley and Wistar Rats in Immunotoxicology Endpoints

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The two most commonly used strains of rat, Sprague-Dawley and Wistar, were assayed to determine possible differences between the strains for major immunotoxicology endpoints: immunophenotyping (IPT), T cell dependent antibody response (TDAR) and peripheral blood cytokine detection. For IPT analysis, whole blood from each rat strain was stained with a cocktail of antibodies to assess Total T, Helper T, Cytotoxic T, B, and Natural Killer cells. Results showed small differences between relative percentages of Total T, Helper T, and B. Cytotoxic T and Natural Killer cells did not display significant differences between the two rat strains. TDAR analysis reflects the literature and illustrates there are no significant differences in the KLH IgG and IgM expression during primary or secondary response between strains. Analysis of peripheral cytokines in rat serum and plasma was assessed. Whole blood from 15 animals per sex was used to create three pools of whole blood per strain. The whole blood pools were stimulated with mitogens to generate cytokine positive plasma, which was spiked into naïve serum or plasma to generate cytokine positive sample. Overall the cytokine expression between the strains was similar. In summary, the strain comparison for these major endpoints is interpreted to indicate that the two strains of rat perform similarly for immunotoxicology monitoring in standard preclinical safety assessment studies.

2516 Understanding Appropriate Use of Cytokine Release Assay Formats as Related to Therapeutic Agent Mechanism of Action

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Following adverse (cytokine storm) events associated with administration of TGN 1412 (anti-CD28 superagonist) in healthy individuals, *in vitro* cytokine release assays (CRAs) are now routinely used as risk assessments to understand potential hazards associated with therapeutic agents prior to first-in-human (FIH) clinical studies. The challenge in designing CRAs is modeling an *in vitro* assessment that has *in vivo* predictive value and accurately reflects risk of cytokine release syndrome (CRS) within the clinic. Despite an industry-wide focus on CRA optimization over the past decade, there is no consensus on preferred assay formats, likely due to the complexity of understanding mechanism of action (MoA) of therapeutic agents while trying to model *in vivo* conditions within a well-controlled *in vitro* assessment. This investigation aims to provide an understanding of the assay format as it related to the MoA of controls and comparator molecules within multiple assay formats including liquid phase and solid phase (wet coat and dry coat). Specifically, anti-CD3 monoclonal (OKT3), anti-CD28 monoclonal (ANC28.1), anti-CD20 monoclonal, and anti-TNF monoclonal antibodies were chosen for evaluation. Additionally, the use of two different serum-free medias were investigated for use in liquid phase CRAs to determine if robust assessments can be accomplished in the absence of fetal bovine serum (FBS). Use of serum-free media compared to complete media containing FBS impacted cytokine production including background (unstimulated) and isotype control cytokine production. These assessments highlight the importance of understanding biologic conditions modeled by *in vitro* assessments, including the impact of media composition on the health and status of immune cells. Additionally, this assessment of serum-free media underscores the importance of evaluating cytokine data as a function of both concentration and fold change (over background or isotype control values). Overall, this poster will focus on the importance of selecting appropriate assay format as related to MoA of therapeutic agents, assay conditions and data presentation and interpretation to provide insight into improving predictability of CRAs.

2517 T Cell-Dependent Antibody Response in Monkey and Rat: Establishing the "Normal Range" in Immune Response for the Assessment of Immunotoxicity

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Increased expectations from regulatory agencies call for additional immunotoxicity tests as part of repeat-dose toxicity studies recommended for the improved prediction potential of adverse effects on the immune system. Evaluation of T-cell-dependent antibody response (TDAR) provides an overall measure of host immune function and requires the active participation of T-lymphocytes, B-lymphocytes, and macrophages/Dendritic cells. Assessment of anti- keyhole limpet hemocyanin (KLH) is fast becoming an industry standard for TDAR assessment. However, there are numerous concerns around the application of TDAR tests, specifically concerning KLH-TDAR assessment, including high animal to animal variability, a lack of an established normal range of the immune response and ambiguity of the level of inhibition of the TDAR to be considered toxicologically imperative. As such, there is an immediate need to evaluate these issues. The objective of this study was to establish a consensus on the implications of these factors on using the KLH-TDAR results in hazard/risk identification, and to establish standard criteria in rodent and non-human primate (NHP) *in vivo* models for immunotoxicants classification. To establish a standard 'normal range', the serum levels of anti-KLH and IgM antibodies titres in naïve Rat and Cynomolgus Monkey were assessed using a validated enzyme-linked immunosorbent assay (ELISA) method following dosing with 300 µg/kg KLH. Animals were administered KLH at the start of the study to induce an initial immune response and given a consecutive dose approximately 2 weeks later to enable a secondary immune response. Blood samples were obtained prior to KLH administration and subsequently throughout the study to monitor anti-KLH response. Response to KLH was calculated using the Area Under the Curve (AUC). Next, correlations between KLH response, key pro-inflammatory cytokine levels (IL-2, IL-6, IL-8, TNFα IFNγ and IL-10) and the number of circulating lymphocytes (CD3+, CD4+, CD8+ and CD14+) were assessed to further develop the 'normal' TDAR model. Taken together, the results from the present study will assist in a more accurate means to classifying changes outside of a 'normal' humoral immune response. The addition of a set range of immune parameters, including cytokines levels and lymphocytes counts will help better define set criteria for KLH-TDAR response in rodent and NHP *in vivo* models commonly used in Immunotoxicology.

2518 Upregulation of ROS Detoxification Genes Protects against Lipopolysaccharide Toxicity: Role of NFkappaB

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Dysregulated response by the body towards an infection leads to tissue and organ damage is known as sepsis. Currently, approximately 18 million people worldwide are affected by this deadly disease annually. However, the mechanism underlying this illness remain not been fully defined. Lipopolysaccharide (LPS), also known as endotoxin, is commonly used in controlled experiments to trigger the symptoms of sepsis. This study was undertaken to determine whether the LPS-induced inflammatory injury could be ameliorated via the

endogenous upregulation of antioxidant defense. CDDO-Im, a novel triterpenoid compound, was used to upregulate endogenous antioxidant defenses. *In vivo* mice trials indicated that pro-inflammatory cytokine levels of TNF-alpha, IL-6, and IL-1β in hepatic tissue were significantly decreased as a result of CDDO-Im treatment in LPS-induced mice. Also, LPS-induced increase in expression of pro-inflammatory cytokine levels is reduced by CDDO-IM in macrophages differentiated from ML-1 monocytes. Additionally, CDDO-IM has been shown to protect against LPS-induced cytotoxicity in macrophage. Interesting, NF-kB transcriptional activity was also noted to decrease upon treatment with CDDO-IM in macrophages. This data demonstrated that the endogenous upregulation of a multitude of antioxidants by CDDO-Im attenuated LPS-induced inflammation and injury. This study may contribute to the advancement of our understanding of treating life-threatening inflammatory diseases such as sepsis.

PS 2519 The Involvement of Lymphocyte-Specific Protein Tyrosine Kinase (LCK) in the Aryl Hydrocarbon Receptor (AhR)-Mediated Suppression of the IgM Response in Human CD5$^+$ Innate-Like B Cells

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LCK is a well-characterized tyrosine kinase critical for cell activation and proliferation. The function of LCK has been extensively investigated in T cells; however, little is known about the role of LCK in B cells. Studies have demonstrated that CD5$^+$ Innate-like B cells (ILBs), a subpopulation of mature B cells, highly express LCK. Previous studies from our laboratory also demonstrated the involvement of LCK in the suppression of the IgM response to AhR activation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in total human B cells. These observations suggested that different subsets of B cells expressed different levels of LCK and had differential sensitivity toward AhR activation. Therefore, we hypothesized that the AhR-mediated suppression of the IgM response involved an increase in LCK activity in CD5$^+$ ILB. In the current studies, we investigated the role of LCK in the context of AhR-mediated suppression of the IgM response by CD5$^+$ ILBs. We demonstrated that AhR activation resulted in a significant increase in LCK, which correlated with significant suppression of the IgM response in CD5$^+$ ILBs. In addition, LCK inhibitor treatment prevented the AhR-mediated suppression of the IgM response in CD5$^+$ ILBs. Furthermore, treatment with an AhR antagonist also prevented the increase of LCK in CD5$^+$ ILBs following AhR activation. Collectively, these data demonstrated a critical role by LCK in the suppression of IgM responses in CD5$^+$ ILBs. *Support by P42 ES00491.*

PS 2520 AhR Activation Enhances Influenza A Virus Gene Expression in Dendritic Cells

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Influenza A viruses (IAV) cause severe disease in humans and are a significant public health problem. Epidemiology studies have shown that human exposure to dioxins and PCBs exacerbate influenza and other respiratory diseases. These compounds bind the aryl hydrocarbon receptor (AhR), and by activating the AhR they hinder effective host response to infection by negatively regulating the ability of dendritic cells (DCs) to prime naive CD8+ T cells. Thus, AhR activation reduces the generation of cytotoxic T cells (CTLs), which are critical for controlling viral infections. Yet, how environmental AhR ligands regulate antiviral defenses in DCs is not fully understood. Recently, transcriptomic analysis of DCs isolated from the lungs of IAV infected mice revealed that AhR activation changed expression of several gene families associated with viral uptake and processing. For example, AhR activation significantly decreased expression of *Cd209a* and *Clec9a*, which encode receptors that detect and bind carbohydrate antigens, influence DC phagocytic activity, and antigen processing pathways. The present study tests the hypothesis that AhR-driven decreased expression of *Cd209a* and *Clec9a* enhances susceptibility of DCs to direct viral infection. To test this, we used *in vitro* derived bone marrow derived DCs (BMDCs) from C57BL/6 mice. Cells were exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to active the AhR, and subsequently infected with IAV. Alterations in viral and endogenous gene expression were measured using qRT-PCR. Influenza virus nucleoprotein (NP), non-structural protein 1 (NS1), and matrix genes were significantly higher in TCDD treated BMDCs compared to vehicle controls. In addition, BMDCs treated with TCDD have significantly increased expression of known AhR target genes *Cyp1a1* and *Cyp1b1*, which indicates sensitivity to AhR activation. These data are further evidence that the AhR is a multifaceted regulator of the immune system, and understanding the effects of its activation will give us further insight into how environmental exposures modify anti-viral immune responses.

PS 2521 Immunologic Assessment of Cannabidiol (CBD) in Human Peripheral Blood Mononuclear Cells (PBMC)

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Cannabidiol (CBD), first discovered in 1940, is one of 113 structurally related cannabinoids in the *Cannabis satvia* plant and is a non-psychotropic phytocannabinoid due to its low binding affinity to cannabinoid receptor (CB1). Recently CBD has garnered much attention for its medicinal properties in the treatment of certain types of epilepsy and as an anti-inflammatory agent. Due to its purported anti-inflammatory properties CBD is being added to skin creams and foods and beverages. The objective of this study was to evaluate pro and/or anti-inflammatory effects by CBD in different cell types present in human PBMC. CBD concentrations extending from 0.001 to 10 μM were added to human PBMC which were then stimulated with either CpG (plasmacytoid dendritic cell (pDC)), LPS (monocytes), anti-CD3/CD28 (CD4 and CD8 cells) or IL-15/21 (NK cells). Phagocytosis, proliferation, activation marker expression and cytokine production were quantified. Out of the 22 unique responses assayed, CBD modulated three responses that were statistically different from their vehicle controls. Interestingly, all three responses were in the monocyte population. Phagocytosis at 10 μM was significantly decreased along with IL-1β and IL-6 production at 10 μM after 24 hours post stimulation with LPS. These studies suggest that CBD preferentially targets certain monocyte-mediated responses while having no effect on the inflammatory responses by pDC or on CD4 or CD8 T cell populations in human PBMC. *Supported in part by GBS Global Biopharma Inc. and Center for Research on Ingredient Safety.*

PS 2522 AhR Controls Expression of the Cell Cycle Regulator Aurora A Kinase in Hematopoietic Stem and Progenitor Cells

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Hematopoietic stem cells (HSCs) self-renew and give rise to all cells of immune system, ensuring lifelong protection against disease. Recent evidence suggests that the environment-sensing transcription factor aryl hydrocarbon receptor (AhR) is an important regulator of HSCs. For example, AhR levels decline as HSCs exit quiescence and actively differentiate, and global loss of *Ahr* (AhR KO) results in an increased rate of HSC proliferation. Yet, the mechanism by which AhR regulates HSCs is poorly understood. To better delineate this, HSCs and multipotent progenitor cells (MPPs) from wild-type and AhR KO mice were isolated by FACS and transcriptome analysis (RNA-Seq) was performed. The most significant differences were seen when analyzing the transition from HSC to MPP in wild type (WT) compared to AhR KO mice, which revealed 1336 differentially expressed genes (DEGs). These DEGs included lineage specific markers such as *Gata1*, *Itga2b*, and *Spi1* (PU.1). Further analysis of the 640 upstream regulators associated with this altered gene expression profile revealed 19 unique regulators in AhR KO compared to WT. Absence of AhR also altered 24 genes involved in cell cycle progression, including the known AhR target gene *Cdkn1a* (p21). A novel component identified in this transcriptomic analysis was Aurora A kinase (*AurA*), which was increased in AhR KO compared to WT HSC and MPPs. AurA is required for mitotic entry into G2 and cell cycle progression. Also, we identified 3 putative AhR regulatory elements in the upstream regulatory region of *AurkA,* suggesting it may be controlled directly by AhR. Similar to AhR KO mice, conditional deletion of the *Ahr* from hematopoietic cells increased AurA levels in HSCs and MPPs. Also, *in vivo* AhR antagonism using CH-223191 increased levels of AurA in HSCs and MPPs. Induction of HSC cycling using 5-fluorouracil (5-FU) increased AurA levels in HSCs and all MPP subsets; AhR activation altered this effect of 5-FU on AurA levels in HSCs and the MPP subsets. Overall, these data highlight a potential novel mechanism by which AhR controls HSC homeostasis, by tightly constraining mitotic progression via influencing Aurora A kinase. These findings advance our knowledge of external environmental signals act through the AhR to perturb hematopoietic stem and progenitor cell function.

2523 Daily Ascending Dosing to Mitigate Cytokine Release Syndrome Induced by an Anti-GPC3 T Cell Redirecting Antibody in Cynomolgus Monkeys

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A CD3 bispecific construct is an emerging measure of cancer immunotherapy, but CD3 bispecific constructs have difficulties in management of adverse effects in clinical use. Cytokine release syndrome (CRS), a potentially life-threatening systemic inflammatory response associated with elevated levels of circulating cytokines, is the most common adverse effect associated with the cancer immunotherapy using this type of constructs. A combination of intra-patient/animal dose escalation and treatment with corticosteroid was reported to reduce CRS, which may be the most effective measure to CRS in the current knowledge. However, this mitigation effect is limited with premedication alone, suggesting that the dosage regimen itself might be a more important factor in reducing CRS. The purpose of this study is to examine how effectively a modified dosing regimen can mitigate CRS. An anti-glypican-3 (GPC3) T-cell redirecting antibody, ERY22, can bind to CD3 and GPC3 in both humans and cynomolgus monkeys. In this study, ERY22 was intravenously administrated to cynomolgus monkeys at the doses of 10 (n=2), 100 (n=1), or 1000 μg/kg (n=1) in single dose administration. In ascending dose administration, ERY22 was intravenously administrated to 2 animals with daily ascending of 1, 3, 10, 30, 100, 300, and 1000 μg/kg during Days 1-7, followed by two shots of 1 mg/kg after one week intervals at Days 14 and 21. Toxicological parameters related to CRS were compared after single or ascending dose with intravenous administration of ERY22. The daily ascending dosing up to 1000 μg/kg markedly reduced the peak cytokine levels as well the clinical severity of CRS whereas single dose with 1000 μg/kg induced severe CRS compared with a single dose of 1,000 μg/kg. Peak cytokine levels following the single and ascending doses were 60,095 pg/mL and 1,221 pg/mL for IL-6; 2,219 pg/mL and 42 pg/mL for IL-2; 353 pg/mL and 14 pg/mL for TNF-α; and 123 pg/mL and 16 pg/mL for IFN-γ. The tolerance acquired with daily ascending doses up to 1,000 μg/kg remained effective for the following weekly doses of 1,000 μg/kg. These results can provide useful information to be extrapolated for human, and will support future applications for CD3 bispecific constructs.

2524 Validation of a 14-Color Flow Cytometry Panel for GLP-Compliant Blood Cell Subpopulation Analyses in the Nonhuman Primate

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Due to the lack of both specific guidelines and adapted standardized cellular quality controls (QC), the validation of a GLP-compliant flow cytometry-based method is a challenging process. Following recommendations from literature and white papers, we developed and validated a 14-color panel (CD3, CD4, CD8, CD20, CD14, CD25, CD69, CD335 + CD159a, CD95, CD28, CD27, CD107a, KI67 and FoxP3) for whole blood from nonhuman primates to be used as a decision making tool within nonclinical toxicology studies. A dual platform approach was applied using a hematology analyzer (ADVIA 2120, Siemens) in combination with a three-laser flow cytometer (MQ16, Miltenyi Biotec) in order to express results in relative (%) and absolute (cells/μL of blood) counts as well as in fluorescence intensity units (MFI) for KI67, CD69 and CD107a. The validation parameters assessed were: precision (intra-, inter-assay), sensitivity (LLOQ), stability [before (BP) and after (AP) process and combined (BP+AP)], robustness (inter-analyst variability) and carryover. Due to cross reactivity between humans and nonhuman primates of the different antibodies, ImmunoTrol cells from Beckman Coulter (stabilized human erythrocytes and leukocytes) were characterized (in terms of acceptance range and stability) in order to be further used as a QC for in-study sample analysis. After optimization (including antibody titration, red blood cell lysis, instrument setting and gating strategy definition), validation results showed that intra- and inter-assay precision values (CV%) ranged from 0.4% to 13.9% and from 0.8% to 26.5%, respectively. LLOQs were determined as up to 30 events for rare subsets (among an average of 220 000 lymphocytes acquired). Stability (expressed as bias % from T_0) was ≤20% for 73, 101, 83 and 73 out of the 120 measured parameters at 4hBP, 6hAP, 24hAP and 4hBP+16hAP, respectively (with a maximum of 148.2%, 32.5%, 50.4% and 93.9%, respectively). There was no significant impact on manual subset gating across two different analysts (CV% ranged from 0.5% to 20.3%). There was no significant inter-sample contamination (≤1%). Despite being unsuitable for intra-nuclear markers, reference ranges for extracellular markers and stability period could be defined for Immuno-Trol. This quasi-quantitative flow cytometry method was thus considered as successfully validated for its intended use in nonclinical GLP studies.

2525 An *In Vivo* PBMC Humanized Mouse Model for Determining Checkpoint and Bispecific Antibody Treatment Related Cytokine Release Syndrome

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Although antibody and CAR-T cell therapies have been successfully used for cancer treatment, they can cause significant adverse effects such as cytokine release syndrome (CRS) in patients. The animal models and *in vitro* human PBMC assays presently in use do not reliably predict the occurrence of CRS in patients unfortunately. This significant gap between pre-clinical testing of novel therapeutics and clinical trials suggests that there is a critical need for translational protocols that could predict immune toxicity more accurately. We have developed a sensitive and reproducible humanized mouse model that can rapidly measure cytokine release *in vivo*. NSG mice and derivative strains were engrafted with human PBMCs for 6 days before being treated with various antibodies including bispecifics, checkpoint inhibitors such as pembrolizumab, avelumab, anti-CD28 and anti-CD3 (OKT3). Cytokine levels in the peripheral blood were determined at 6 hours post dose. The cytokine release detected from this model is both donor-dependent and drug-specific for cytokine and toxicity response. NSG mice lacking murine MHC Class I and Class II provides a useful tool to study CRS in the absence of GvHD. This PBMC humanized mouse model was applied to examine cytokine release from novel bispecific antibodies treatment. A dose- dependent cytokine release was detected from a BCMAxCD3 bispecific antibody in PBMC humanized Mice. Significant higher cytokine release was observed in a high-affinity bispecific antibody compared to cytokine release from a low-affinity bispecific antibody, which was correlated to different mice clinical symptoms and to downstream readouts. In summary, we have developed a novel PBMC humanized mouse model that is capable of differentiating human PBMC donors based on response to human antibody *in vivo*. Our newly developed *in vivo* CRS assay may be used to assess *in vivo* safety of various human therapeutic antibodies including immune checkpoint inhibitors and bispecifics with better reliability than an *in vitro* assay. It may also have potentials for the safety assessment of CAR-T therapy in drug development.

2526 Anandamide Impaired Immune Cell Activation, Induced Apoptosis, Inhibited T Cell Proliferation, and Promoted Induction of MDSCs and T Regulatory Cells following Treatment with Staphylococcus Enterotoxin B (SEB)

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Staphylococcus Enterotoxin B (SEB) produced by *Staphylococcus aureus* causes a wide range of diseases including acute lung injury, multiple organ failure and may be fatal. SEB is a superantigen that activates up to 30% T cells by crosslinking the T cell receptor (TCR) to nonpolymorphic region of MHC class II on antigen presenting cells (APC). SEB is a CDC select agent of bioterrorism. Anandamide (AEA), an endogenous cannabinoid, is part of endocannabinoid system (ECs) and binds to CB1 and CB2 receptors. In the current study, splenocytes were pretreated with AEA (20uM) or vehicle (VEH) and SEB 1ug/ml was used to activate the lymphocytes *in vitro*. Flow cytometric analysis showed that there was a significant decrease in the inflammatory cells including activated immune cells based on expression of biomarkers such as CD69 and CD44 to assess activation in SEB+AEA group when compared to SEB+VEH group. VEH-treated control group showed no activation. In addition, CD4+T cells, CD8+ T cells, Vβ8+ T cells and NK-T cells were also decreased in SEB+AEA group compared to SEB+VEH group. Furthermore, flow cytometry data showed an induction of CD11b+Gr1+ Myeloid Derived Suppressor Cells (MDSCs) and T regulatory cells (CD4+FOXP3+). There was also a significant decrease in the proinflammatory cytokines such as IL2 and IL6 as assessed by ELISA. We next examined the role of AEA in SEB-induced T cell proliferation through the ^{3}H-thymidine incorporation assay and found that there was a significant decrease in the proliferation in SEB+VEH compared to SEB+AEA group. Apoptosis assay was performed wherein we found a significant increase in apoptotic cells in SEB+AEA compared to SEB+ VEH group. In addition, we determined the expression of anti-inflammatory genes by RT-PCR

and found that NOS2, Arg1 and FOXP3 were upregulated that correlated with MDSCs and T regulatory cells in SEB+AEA compared to SEB+VEH group. In summary, AEA plays a protective role against SEB by inducing apoptosis in immune cells, inhibiting T cell proliferation, as well as by promoting anti-inflammatory MDSCs and T regulatory cells. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.*

2527 *In Vitro* Differentiation and Characterization of Plasmablasts and Plasma Cells from Cynomolgus Macaque CD20+ B Cells

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Assessing biomarkers for B cell targeted therapies in nonclinical studies is hampered by low circulating levels of plasma cells, scarcity of cross-reactive reagents for cynomolgus monkeys, and a general lack of understanding of B cell development in nonhuman primates. We identified conditions to differentiate monkey CD20+ B cells into antibody-producing plasma cells (PCs) with a three-phase culture system beginning with an antigen-independent stimulation of CD20+ B cells by CpG (phase 1) (Huggins et al, 2007), followed by plasmablast expansion (phase 2), and ending with the terminal differentiation of short-lived plasma cells (phase 3). We confirmed differentiation with a combination of morphological, flow cytometry, and immunoglobin (Ig) secretion assays. Cytospins were prepared throughout the time course of differentiation and cell morphology was evaluated after May-Grünwald/Giemsa staining and CD138 immunohistochemical labeling. CD138 positive cells with eccentric nuclei and prominent Golgi apparatus, consistent with features of plasmablasts (PBs) and PCs, were detected from cells in culture by the end of Phase 2. A flow cytometry panel for identification of cynomolgus monkey PBs and PCs was developed using peripheral blood and bone marrow with the commonly used human PC marker of CD138, HLA-DR, and the transcription factor IRF4 (Shaw et al, 2017). Monkey PBs were identified as CD138-/IRF4+/HLA-DR^{high}, while PCs were primarily found to be CD138+/IRF4+/HLA-DR$^{neg/low}$. Using this flow cytometry panel, we were able to show a gradual loss of surface marker CD20 and HLA-DR and a gain of CD138 in the differentiating cells, consistent with the phenotype of cells progressing towards a PC fate (Robillard et al, 2014). Supernatants collected for quantitation of IgG by ELISA at the end of each culture phase had increasing amounts of IgG over the course of the experiment, with maximal IgG production at Phase 3. Cell sorting using the markers on the flow panel, evaluation of additional Ig subsets, and assessment of cytokine production by differentiated single cells via ELISPOT is ongoing. Our results indicate that CD20+ B cells isolated from whole blood of cynomolgus macaques can differentiate into PBs and PCs *in vitro* and that the combination of HLA-DR, IRF4, and CD138 are useful in identifying monkey PBs and PCs.

2528 Translational Investigation of the Intrinsic Immunogenicity of Tolvaptan and Metabolites through the Use of *In Vitro* Cell Culture Platforms

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Tolvaptan DILI has been of interest since the observation of an imbalance in hepatic safety signals between tolvaptan and placebo in the TEMPO 3:4 (NCT00428948) trial. The clinical pattern of such DILI includes a delayed reaction onset and rapid recurrence of injury upon re-challenge, indicating a role of the adaptive immune system in the manifestation of liver injury [1]. Hence, this study assessed the intrinsic immunogenicity of tolvaptan in healthy donors using *in vitro* T-cell culture platforms and evaluated the translational relevance to (ADPKD) subjects enrolled on the TEMPO 3:4 (NCT00428948) trial whom experienced DILI. Peripheral blood mononuclear cells (PBMC) derived from 3 healthy donors were primed to tolvaptan and 2 major metabolites; DM-4103 and DM-4107. T-cell clones (TCC) from healthy donors exhibited differential cross reactivity profiles between antigens depending on the compound originally primed to. A signature core secretory molecule profile for TCC was identified via ELISpot, with IFN-gamma, IL-13, granzyme B and perforin featuring almost universally upon antigen challenge. CD4+ TCC were found to be HLA-DR restricted with allelic dependence identified. Mechanistically, compound-specific TCC activation was dependent on the presence of soluble drug rather than antigen-pulsed APCs and could occur within 4 hours of antigen exposure. DM-4107-responsive TCC were also identified from PBMC of patients that experienced liver injury, and expressed similar phenotypes to those generated from healthy donors. Tolvaptan DILI may be attributable to an adaptive immune attack upon the liver. Data from this study implicates DM-4107 as a major antigenic determinant, which, alongside the possibly altered disposition of this metabolite under ADPKD disease state, and the greater therapeutic dose for this indication, alludes to why tolvaptan DILI is exclusively observed in this therapeutic indication. [1] Watkins PB, Lewis JH, Kaplowitz N, Alpers DH, Blais JD, Smotzer DM, *et al.* (2015). Clinical Pattern of Tolvaptan-Associated Liver Injury in Subjects with Autosomal Dominant Polycystic Kidney Disease: Analysis of Clinical Trials Database. *Drug Safety.* 38**:** 1103-1113.

2529 The Importance of Dose Metrics in Predicting the Incidence of Percutaneous Immediate-Type Hypersensitivity

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For the effective assessment of human health risks, appreciating the dose metrics that is correlated with the incidence of toxicity is necessary. Although the incidence of delayed-type hypersensitivity (skin sensitization) is correlated with antigen dose per unit area, the dose metric which correlates with incidence of percutaneous immediate-type hypersensitivity (antigenicity) remains unclear. Several animal tests were conducted to clarify the dose metrics for predicting the incidence of percutaneous immediate-type hypersensitivity. Papain, which causes immediate-type hypersensitivity via percutaneous sensitization in humans, was used to sensitize guinea pigs. Total dose per animal or dose per unit area was adjusted to understand the conditions that contribute to sensitization. We collected sera from sensitized guinea pigs to perform passive cutaneous anaphylaxis (PCA) and ELISA for papain specific IgG1. To elucidate the underlying mechanism that contribute to the dose metrics for immediate-type hypersensitivity, Alexa488-labeled papain was administered to CBA/J mice in varying total doses per animal or doses per unit area. The number of antigen-bearing B cells (Alexa488+/B220+) in the draining lymph nodes was calculated using flow cytometry. ELISA was performed to quantify papain specific IgG1 and IgE levels in the serum of sensitized mice. In guinea pigs, the PCA test revealed that positive PCA reaction rates did not correlate with dose per unit area but with total dose per animal. Papain-specific IgG1 levels also correlated with total dose per animal. In CBA/J mice, the number of antigen-bearing B cells in the draining lymph nodes and papain-specific antibody (IgG1 and IgE) levels correlated with total dose per animal. The current study demonstrated that the incidence of immediate-type hypersensitivity correlates with the amount of total dosage per animal. Although the process by which antigens activate dendritic cells in the skin is important for delayed-type hypersensitivity, the process by which antigens reach draining lymph nodes to activate B cells is important for immediate-type hypersensitivity.

2530 Comparative Analysis of Nonhuman Primate Plasmacytoid Dendritic Cell Immunophenotyping in Tissue

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Plasmacytoid dendritic cells (pDC) are one of two main groups of DC described in humans. These antigen-presenting cells are derived from bone marrow precursors and play a key role in regulating immunity through activation of T- and B-lymphocytes, NK cells, and cytokine secretion, thus linking the innate and adaptive immune response. pDC are involved in the antiviral immune response, regulate mucosal immunity and the development of central and peripheral tolerance, and associated with immunopathology of autoimmune and inflammatory diseases. While human myeloid DC (mDC) characterized as HLA-DR+CD11c+CD123-, pDC are HLA-DR+CD11c-CD123+. Non-human primate (NHP) pDC exhibit similar morphology and phenotype to their human counterparts. In humans, pDC are distinguished from mDC by the expression of CD123, CD303 (BDCA-2) and CD304, while CD123 is the only specific marker of pDC available for use in NHP models, due to the lack of cross-reactivity and poor sensitivity of some anti-human antibodies. Since NHPs are one of the main models in drug safety assessment, it is important to identify specific antigens for detection of pDC in tissues in pre-clinical toxicology studies. The goal of this study was to design and develop an immunostaining panel that would allow detection of pDC in NHP tissue through cross-platform comparison of immunophenotyping data obtained by flow cytometry with immunohistochemistry (IHC) and immunofluorescence (IF). Bone marrow and peripheral lymphoid tissue (spleen, tonsil) were collected from naïve cynomolgus monkeys (*Macaca fascicularis*). Immunophenotyping of unfixed tissue leukocytes was performed by flow cytometry and IHC and IF were completed on formalin-fixed paraffin-embedded tissue sections from the same animal. Results demonstrated that CD303 can be used as a specific

marker for pDC detection in NHP tissues by IHC and IF. While CD123 is still one of the critical antigens for identifying pDC via flow cytometry, its expression on endothelial cells makes it a less favorable marker for histopathological evaluation using IHC or IF. Data showed a direct correlation of pDC tissue distribution when measured via flow cytometry and IHC. As pDC constitute ≤ 1% of CD45+ lymphocytes in tissues, results indicate lower pDC proportions in spleen and tonsils compared with bone marrow. Taken together, these results provide a valuable approach for using a specific panel of antibodies for pDC immunophenotyping in tissue sections.

2531 Identification of the Dog Orthologue of Human MAS-Related G Protein Coupled Receptor X2 (MRGPRX2) Essential for Drug-Induced Pseudo-Allergic Reactions

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MAS-related G protein coupled receptor-X2 (MRGPRX2) is a Gi or Gq-coupled receptor expressed in human mast cells and associated with drug-induced pseudo-allergic reactions. Dogs have been reported to be highly susceptible to the drug-induced anaphylactic reactions caused by various agents including fluoroquinolones; however, the distribution and its physiological function of dog MRGPR families including MRGPRX2 remains largely unknown. In the present study, we clarified the distribution of dog MRGPR family genes (X2, D, F, and G) in total of 21 organs/tissues from male dogs (n = 3) including lung and skin by a quantitative PCR method. We also investigated the stimulatory effects of compound 48/80 and several fluoroquinolones (ciprofloxacin [CPFX], gatifloxacin [GFLX], levofloxacin [LVFX], and pazufloxacin [PZFX]) on HEK293 cells transiently transfected dog MRGPR family genes or human MRGPRX2 to identify the physiological function of dog MRGPR family genes by intracellular Ca^{2+} mobilization assay. Amino acid sequence homology of dog MRGPRX2, D, F, or G to human MRGPRX2 was 62, 41, 38, or 32%, respectively. Dog MRGPRX2 and MRGPRG were distributed in the limited organs/tissues including skin and eyelid, whereas MRGPRD and MRGPRF were extensively expressed in almost all organs/tissues examined. Intracellular Ca^{2+} mobilization assay demonstrated that HEK293 cells expressing dog MRGPRX2 or human MRGPRX2, but not dog MRGPRD, MRGPRF and MRGPRG, responded to compound 48/80, CPFX, GFLX, and LVFX. Interestingly, the concentration of compound 48/80 inducing intracellular calcium changes in dog MRGPRX2-expressing cells was comparable to that in human MRGPRX2-expressing cells. In contrast, the concentrations of CPFX, GFLX, and LVFX inducing intracellular calcium changes in dog MRGPRX2-expressing cells were 3- to 27-fold lower than those in human MRGPRX2-expressing cells. The results indicate the functional heterogeneity of dog MRGPRX2 to various histamine releasing agents. PZFX which is known not to induce histamine release in dogs did not change intracellular calcium in dog MRGPRX2- and human MRGPRX2-expressing cells. The present results suggest that dog MRGPRX2 is the functional orthologue of human MRGPRX2 and plays an essential role for drug-induced anaphylactic reactions in dogs.

2532 Host Cell Proteins Induce Inflammation and Immunogenicity as Adjuvants in an Integrated Analysis of *In Vivo* and *In Vitro* Assay Systems

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Host cell proteins (HCPs) are contaminated proteins remaining after purification of biopharmaceuticals. Recent reports revealed clinical implications of HCPs in anti-drug antibody (ADA) development in patients without any inflammatory effects. During preclinical development of an intravitreal biopharmaceutical, we observed ocular inflammation in rabbits which may have been associated with the contamination by HCPs. Therefore, we evaluated the inflammatory effects and immunogenicity of HCPs in an *in vivo* study by intravitreal administration to rabbits and an *in vitro* THP-1 cells assay. *Escherichia coli* HCP antigen concentrate used for the positive control of the *E. coli* Host Cell Protein ELISA Kit was used as HCPs in this study. HCPs at 200 ng/eye with or without the commercial intravitreal biopharmaceutical, ranibizumab at 0.25 mg/eye were administrated intravitreally to rabbits. For *in vitro* examination, differentiated THP-1 cells were stimulated with HCPs at 0.17 to 10.88 μg/mL with or without ranibizumab at 0.2 mg/mL. As a result, co-administration of HCPs and ranibizumab induced ocular inflammation which was characterized histopathologically by mononuclear cell infiltration in the vitreous body, ciliary body, iris, choroid, and perivascular space of the optic disc. Furthermore, ADA was detected in the vitreous fluid by co-administration of HCPs with ranibizumab in rabbits, but not by HCPs alone. The immunostimulatory effects including increases in inflammatory cytokine secretion (IL-1β, IL-8, and MIP-1β) and upregulation of cell surface markers (CD80 and CD54) involved in antigen presentation were also detected in the THP-1 cells assay, and these reactions were enhanced by co-stimulation with ranibizumab. In conclusion, the present study firstly revealed that HCPs induce inflammation and immunogenicity as adjuvants when they are co-treated with biopharmaceuticals in both *in vivo* and *in vitro* systems. This study provides useful data for risk assessment of HCPs and for discussion of local inflammation induced by biopharmaceuticals. Integrated analyses by *in vivo* rabbit models and *in vitro* assay systems using THP-1 cells would be useful to evaluate the immunological risk of HCPs.

2533 Sex Steroid Hormones and Macrophage Phenotype in Nanoparticle-Induced Lung Inflammation

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Respiratory diseases such as asthma and COPD occur more frequently in women compared to men, yet the mechanisms behind this sex-bias are unknown. Our laboratory has reported that this bias also occurs in a murine model of multi-walled carbon nanotube (MWCNT)-induced lung injury. MWCNT-treated female mice develop greater innate lymphoid cell-independent acute type 2 inflammation including eosinophilia, cytokine expression, and airway hyper-reactivity (AhR) compared to male mice. This increased acute inflammation precedes worse chronic lung pathology with greater epithelial cell hyperplasia and centriacinar MWCNT accumulation in females. Therefore, we hypothesize that sex-steroid hormones, specifically estrogen receptor alpha (ERα) signaling, promotes an exaggerated M2a phenotype in alveolar macrophages (AMs) and subsequent type 2 inflammation in MWCNT-exposed females. Two mouse models were used to test this hypothesis. First, in order to eliminate the majority of sex-steroid hormone production, female C57BL/6 mice were allowed to develop normally and then ovariectomized (OVX) at 8 weeks of age. Second, intact female mice were implanted with osmotic pumps containing an ERα antagonist to specifically block endogenous ERα signaling. OVX and antagonist-treated mice were then exposed to MWCNTs via oropharyngeal aspiration (2 mg/kg body weight) and sacrificed 7 days later. Lung lavage fluid (LLF) was collected for high-sensitivity cytokine measurement and flow cytometry analysis of immune cell recruitment. AMs were isolated from the LLF by adherence and phenotype was determined by 24-hour *ex vivo* culture and measurement of cytokines released. The AM-specific cytokine profile was compared to that of the LLF and used to determine functional AM phenotype and inflammatory signaling. Pulmonary function outcomes were also determined through lung resistance and dynamic compliance measurements. Results from OVX and antagonist-treated mice will be compared to that of normal, intact females; changes in immune cell recruitment, cytokine expression, AM phenotype, and AhR to define the role of sex-steroid hormones in particle-mediated inflammation and the mechanism of female-biases in lung disease.

2534 Ethanol Dysregulates Chondrocyte Differentiation via Different Sources of Reactive Oxygen Species in Chondrocyte ATDC5 Cells

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Alcohol abuse is a well-known risk factor for osteoporosis. Skeletal toxicity associated with ethanol (EtOH) exposure is characterized by oxidative stress mediated suppression of bone formation, stimulation of bone resorption and dysregulation of chrondrocyte differentiation in the growth plate resulting in shorter osteopenic long bones. EtOH can produce reactive oxygen species (ROS) as the result of several different pathways including CYP2E1, NADPH oxidases (NOX) and secondary to mitochondrial injury. However, endogenous ROS signaling may also play an important role in normal bone cell differentiation. The current study was designed to examine the effects of EtOH (0/25/50 mM) in the presence or absence of the dietary antioxidant and glutathione precursor N-acetylcysteine (NAC) (0.1/1 mM); the mitochondria targeted antioxidants MitoQ and MitoTEMPO (50/500 nM) or the NOX4 inhibitor GKT137831 (0.1/1 μM) on *in vitro* differentiation, cartilage formation and mineralization in the embryonic chondrocytic rat ATCD5 stem cell line. ATCD5 cells were grown to confluence and exposed to chondroblastogenic medium for 12-14 d to assess cartilage formation by Alcian blue staining and aggrecan (Acan) mRNA expression of mRNAs encloding Col2a1 and Col10a1 as indicators of chondrocyte differentiation. EtOH suppressed chondroblastogenesis and cartilage formation dose-dependently ($P<0.05$). However, this process

was unaffected by GKT137831. Moreover, high doses of mitochondrial ROS inhibitors and NAC inhibited this process in the absence of EtOH. In contrast, assessment of mineralization by Alizarin Red staining at 16 d revealed EtOH dose-dependently stimulated this process ($P<0.05$). MitoQ and MitoTEMPO appear to block EtOH effects on mineralization but NAC exacerbated EtOH effects. These data suggest EtOH dysreguates chondroblastogenesis while stimulating mineralization of embryonic chondrocytic cells processes consistent with dysregulation of bone growth plate formation *in vivo*. EtOH effects on chondrocyte differentiation appear ROS independent and endogenous ROS signaling appear to regulate this process. In contrast EtOH-stimulated mitochondrial ROS appears to play a role in stimulation of mineralization. *Supported in part by NIAAA R37 AA018282 (M.J.R.) and NSF grant #1659752.*

2535 Role of NOX4 Expression in Osteoblast-Precursors in the Development of Alcohol-Induced Osteopenia

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Alcohol abuse is a well-known risk factor for skeletal toxicity and the development of osteoporosis. Ethanol (EtOH) has been demonstrated to inhibit bone formation and stimulate bone resorption. However, the molecular mechanisms underlying alcohol-induced osteopenia remain the subject of active investigation. Whole-body knockout of NOX4 in mice has demonstrated that reactive oxygen species (ROS) signaling via the NADPH oxidase enzyme NOX4 appears to play a role in regulation of endogenous bone turnover and EtOH actions. To establish whether NOX4 expressed locally in osteoblast precursors mediate these effects, we have generated pre-osteoblast-specific Prx-Cre-Lox NOX4 conditional knockout mice where the enzyme is oblated specifically in osteoblast precursor cells in long bones and exposed male and female Cre-Lox and NOX4 fl/fl mice to a high fat Leiber-DiCarli liquid diet with or without replacement of up to 28% of food calories with EtOH for 90 days. The hypothesis is that ROS produced in these cells by NOX4 play a significant role in the development of alcohol-induced osteopenia. To test this, the effects of EtOH were determined on bone microstructure of tibias and by femur shaft mRNA expression. In the cortical region of the tibia, the Prx-NOX4 Cre-Lox mice had overall decreased bone area ($p = 0.038$). In mice with the EtOH diet, there was a decrease in bone area and cortical thickness ($p < 0.001$ & $p=0.001$, respectively) independent of genotype. Results from femur shaft mRNA confirms that mRNA NOX4 expression is decreased by more than 95% in Prx-NOX4 Cre-Lox mice ($p < 0.001$) while RANKL, which controls bone reabsorption, is not affected by EtOH or NOX4 in this model. These data demonstrate that while the bone microstructure differs between males and females, EtOH and NOX4 independently affect the cortical bone region. *Supported in part by NIH NIAAA R37 AA018282 (MJR) and LSUHSC Department of Genetics.*

2536 Nrf2 and CSE as Critical Molecules in Parallel Pathways for Repression of Xenobiotic-Mediated Electrophilic Stress in Mice

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Transcription factor NF-E2-related factor 2 (Nrf2) plays a key role in detoxification of electrophiles via formation of glutathione adducts and subsequent excretion into extracellular spaces. We found that reactive sulfur species, such as cysteine persulfides produced by cystathionine γ-lyase (CSE), capture environmental electrophiles through formation of sulfur adducts. However, contributions of Nrf2 and CSE to the blockage of environmental electrophile-mediated toxicity remain to be evaluated. The aim of this study was to clarify roles that CSE and Nrf2 play in the protection against various environmental electrophiles. We also wished to clarify the molecular basis of the developmental window of toxicity through investigating expression levels of Nrf2, reactive sulfur species-producing enzymes and sulfur nucleophiles during developmental stages of mice. Methods: Wild-type, CSE knockout (KO), Nrf2 KO, Nrf2/CSE double KO (DKO) mice, and their primary hepatocytes were analyzed in this study. Methylmercury (MeHg), Cadmium, 1,4-naphthoquinone, crotonaldehyde, and acrylamide were used. We conducted Western-blotting, real-time PCR, MTT assays, LC-ESI-MS/MS analysis, ALT activity, histopathological analysis, and rotarod test. Primary hepatocytes from DKO mice were significantly more sensitive to the environmental electrophiles than each single KO counterpart. Both Nrf2 and CSE single KO mice were highly susceptible to MeHg and cadmium, and such sensitivity was further exacerbated in the DKO mice. Lower level expressions of CSE and sulfur nucleophiles than those in adult mice were observed in a window of developmental stage. Our mouse model provided new insights into the response to environmental electrophiles; while Nrf2 is recognized as a key transcription factor for detoxification of environmental electrophiles, CSE is crucial factor to repress their toxicity in a parallel mode. In addition, the sensitivity of fetuses to MeHg appears to be, at least in part, associated with the restricted production of reactive sulfur species due to low-level expression of CSE.

2537 Nrf2 Deficiency Ameliorates Thioacetamide-Induced Liver Injury in Mice via Accelerating Degradation of CYP2E1

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Nrf2 is a master transcription factor in anti-oxidative and anti-electrophilic stress, with controversial reports on its role in chemical toxicity. In the present study, we investigated the role of Nrf2 in thioacetamide (TAA)-induced hepatoxicity and underlying mechanisms. Male *Nrf2*-knockout (*Nrf2*-KO) and *Nrf2*-wild-type (WT) mice were intraperitoneally administrated with TAA subacutely with escalating dosing regimen (30, 100, 200, 300 mg/kg, 5 weeks) or acutely (one injection of lethal dose 50 mg/kg or non-lethal dose 30 mg/kg). In subacute experiment, *Nrf2*-KO mice were resistant to TAA-induced liver fibrosis according to sirrus red staining, collagen deposition and expression of fibrosis markers. *Nrf2*-KO mice showed a higher survival rate than WT mice in response to the lethal dose of TAA. Acute liver injury characterized by histological analysis, and serum levels of alanine aminotransferase and aspartate aminotransferase, was ameliorated in *Nrf2*-KO mice compared with WT mice. Hepatic mRNA levels of *Gclc*, *Nqo1* and *Cyp2a5* were lower, while the content of malondialdehyde was higher in *Nrf2*-KO mice than WT mice after acute or subacute TAA treatment. Proportion of oxidized TAA metabolites in the urine and expression of TAA metabolic marker acetyllysine were significantly lower in *Nrf2*-KO mice than WT mice after acute TAA administration. *Nrf2* deficiency aggravated TAA-induced decrease in hepatic CYP2E1 protein levels. In addition, CYP2E1 protein levels were significantly lower, while the intracellular levels reactive oxygen species were significantly higher, in *Nrf2*-KO primary mouse hepatocytes than WT hepatocytes with TAA treatment (20 mM, within 12 h). Protein degradation of CYP2E1 was accelerated in Nrf2-KO hepatocytes. Our findings suggest that *Nrf2* deficiency prevents from TAA-induced liver injury by abrogating CYP2E1-mediated metabolic bioactivation. Oxidative stress resulting from *Nrf2* deficiency may impair protein stability of CYPTE1. This finding has significant implications in toxicity of chemicals that are bioactivated by CYP2E1.

2538 Imaging-Based Single Cell Dynamics of KEAP1/Nrf2 Pathway Activation Status to Quantify Pro-Oxidant-Induced Cell Fate Decisions

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A common mode of action (MoA) involved in drug induced liver injury (DILI) are the formation of reactive oxygen species and radical metabolites. This is exemplified by drugs causing intrinsic DILI like acetaminophen but is also suspected to occur with drugs causing idiosyncratic DILI. The Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is essential for the cellular defence against such cell damage by orchestrating the oxidative stress response (OSR), i.e. by activation of glutathione synthesis, the thioredoxin system and sulfiredoxines. We want to better understand the dynamics and amplitude of the Nrf2 pathway activation in drug induced liver injury as it may give insights for the liver toxicity liability of chemicals. Here we present a novel approach that allows the assessment of the interplay between the activation of the Nrf2 pathway and cell fate decisions (adaptive vs adverse response). Thus, we created a dual-color HepG2 fluorescence reporter cell line (Nrf2-GFP and Srxn1-mScarlet) to follow the translocation of the transcription factor Nrf2 to the nucleus and the subsequent activation of the transcriptional response by sulfiredoxin 1 (SRXN1) on a single cell level. Through single cell tracking we followed the cellular response upon the exposure to a set of compounds inducing oxidative stress and radical formation. Furthermore, through the preexposure to the Nrf2 activator Bardoxolone methyl (CDDO-me) and the L-buthionine sulfoximine (BSO), an effective inhibitor of glutathione synthetase, we simulated pre-activated state or inhibited state of the OSR. A siRNA knock allowed the biological simulation of the inhibition and activation of the OSR. The dynamical patterns found in the cells under the various condition allowed to judge when an exposure to a compound becomes critical for a cell and subsequently lead to an adverse outcome if correctly extrapolated to the *in vivo* situation. This detailed characterization of compounds might add great value towards the correct prediction of DILI potential *in vitro* and if integrated with other MoA driven test systems to an alternative to preclinical

safety testing *in vivo*. *This work was part of the EU-ToxRisk project and received funding of the European Union's Horizon 2020 research and innovation program under grant agreement No 681002.*

2539 Glutathione Depletion and Nrf2 Activation Underlie PFOS Toxicity in Human Kidney Cells

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Perfluorooctanesulfonic acid (PFOS), a persistent xenobiotic historically found in firefighting foams and various consumer products, is known to activate Nuclear Factor(erythroid-derived) like factor 2 (Nrf2), a transcription factor that regulates cellular antioxidant defense genes. Nrf2 activation is also hypothesized to be mediated by glutathione (GSH), the predominant cellular redox buffer. We interrogated whether PFOS activates Nrf2 by inducing reactive oxygen species (ROS) or via GSH depletion. We used a DCF assay to quantify ROS levels and a human Nrf2-GFP fusion protein to visualize Nrf2 dynamics in live Human Embryonic Kidney (HEK293T) cells. This noncancerous cell line conserves Nrf2 signaling in human kidney, a known PFOS target organ. Exposure to model prooxidant and ROS 50 μM tert-butyl hydroperoxide (tBOOH) significantly increased ROS levels at 0.25 and 1 hour. Cells exposed to PFOS showed significantly increased ROS production at 24 hours; 3.2, 6.4, and 64 μM PFOS elicited the greatest changes (n=3 independent experiments). A 24 hour pre-exposure to 100 μM N-Acetyl Cysteine, which bolsters cellular GSH levels, rescued these changes; cells showed decreasing trends in ROS levels starting as early as 1 hour at all concentrations of PFOS. Next, we used time lapse fluorescent microscopy to investigate the effects of these redox disruptions on Nrf2 localization. A 20 minute exposure to tBOOH was sufficient to significantly increase nuclear Nrf2 expression whereas 10 μM tBHQ, a quinone that activates Nrf2, did not significantly increase in nuclear Nrf2 until at least 3 hours. At 9 hours following PFOS exposure, 6.4, 32 and 64 μM PFOS induced a 25% increase in nuclear localization of Nrf2. This change was largely due to a 40% increase in *de novo* expression of nuclear Nrf2 (n= 2 replicates). Next, we assessed the viability of PFOS exposed cells using a Trypan Blue Exclusion Assay. All doses of PFOS induced significantly greater cell death than DMSO controls with 32 μM PFOS inducing 25% more cell mortality (n= 2 independent experiments). Our data indicate that multiple doses of PFOS lead to secondary oxidative stress via GSH depletion. These data indicate GSH depletion induces Nrf2 nuclear translocation, a potential mechanism underlying PFOS toxicity. *This work was supported by R01ES025748.*

2540 Administration of a Pharmacologic Iron Chelator Reduces the Development of Cartilage Lesions in a Model of Idiopathic Osteoarthritis

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Iron is a mineral required for numerous biological processes. While crucial for sustaining life, iron also has the potential to promote tissue damage by participating in reactions that generate free radicals. Despite this, there are no direct iron excretion mechanisms beyond normal turnover of skin and gastrointestinal epithelial cells. Indeed, iron has been implicated in several aging-associated disorders, including neurodegenerative disorders and some types of cancers. While iron has been characterized in these conditions, its role in age-related/primary osteoarthritis (OA) remains relatively unexplored. As the leading cause of adult disability in the United States, OA causes pain, swelling, and overall decreased mobility of affected joints. Unfortunately, the underlying mechanisms driving disease pathogenesis remain poorly understood, and there are currently no treatments available to slow OA once it has started. We hypothesize that iron accumulation in joint tissues throughout aging may contribute to the development of primary OA. Here, we assess the ability of systemic iron reduction—achieved by administration of the pharmacologic chelator deferoxamine (DFO) - to prevent or delay the onset and/or progression of primary OA in an animal model of the disease. All procedures were approved by the University's Institutional Animal Care and Use Committee. Three-month-old male Dunkin-Hartley guinea pigs were randomly assigned to receive either 46 mg/kg of DFO or vehicle control twice daily (n=8 per group). Animals were subjected to open field behavior monitoring once per month throughout the study, with baseline activity levels collected prior to initiating treatment. The study was terminated when animals were 8-months-old, and hind limbs were collected for structural and gene expression analysis of knee joints. Collectively, our results indicate that decreasing systemic iron levels via pharmacologic iron chelation may prevent cartilage deterioration by beneficially modulating local iron levels within the knee. Gene expression analysis showed that treatment with DFO decreased oxidant damage and apoptosis, which maintained the mobility of these animals relative to control. Results from this study indicate that iron may be involved in OA pathogenesis and that use of a pharmacologic iron chelator could hold potential as an intervention for this disease process.

2541 Metabolome-Wide Association Study (MWAS) of Plasma Cystine in Healthy Aging Men

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Cystine (CySS) is an oxidized form of cysteine, abundant in plasma and having different distribution in males and females. Plasma CySS is a measure of oxidative stress and increases with age, but the mechanistic basis is unknown. We hypothesized that if environmental exposures causing oxidative stress in men contribute to increased CySS concentrations with age, a metabolome-wide association study of age should reveal environmental chemical associations with CySS. Perform metabolome-wide association study (MWAS) of plasma CySS in healthy aging men to test for association of environmental chemicals with CySS. Methods: Plasma from 70 men (21 to 90 y; mean, 44.9 y) and without known disease (PRE-MED cohort; NCT00336570) was used. Plasma CySS was quantified by HPLC (mean, 69.2 μM). High-resolution metabolomics of plasma was used for relative quantification of environmental chemicals and metabolites. Samples were analyzed by LC-HRMS with +ESI on C18; data were extracted by apLCMS and xMSanalyzer and log-transformed prior to biostatistics and bioinformatics. Metabolome correlations with CySS and age were performed by xMWAS using Spearman correlation ($r > 0.25$, $P < 0.05$). xMSannotator with HMDB and KEGG was used for metabolome annotation and with the Toxin and Toxin-Target DataBase (T3DB) for environmental chemical annotation. The results showed 183 metabolites positively associated with CySS and 360 positively associated with age ($r > 0.25$, $P < 0.05$). Twenty metabolites including phospholipids and fatty acids were positively associated with both CySS and age. Of those associated with CySS, 19 matched environmental chemicals. These include herbicides, pesticides and insecticides. Of metabolites associated with age, 43 matched environmental chemicals. Correlation of a general herbicide including MCPP (methylchlorophenoxypropionate) and asulam with age was relatively high ($r > 0.4$). The results show that environmental chemicals associate with plasma CySS in healthy aging men. The numbers of environmental chemicals associated with age were greater in men than women. The results indicate that cumulative environmental exposures or decline in elimination of environmental chemicals could contribute to increased oxidative stress with age.

2542 Impact of Sex, Age, and Diet on the Cysteine/Cystine and Glutathione/Glutathione Disulfide Plasma Redox Couples in Mice

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Age, sex and diet are well-established risk factors for several diseases. In humans, each of these variables has been linked to differences in plasma redox potentials (E_h) of the glutathione/glutathione disulfide (GSH/GSSG) and cysteine/cystine (Cys/CySS) redox couples. Mice have been very useful for modeling human disease processes, but it is unknown if age, sex and diet affect redox couples in mice as they do in humans. The purpose of the present study was to examine the effects of these risk factors on plasma redox potentials in C57BL/6J mice. We found that age had no effect on either redox couple in either sex. Plasma E_h Cys/CySS and E_h GSH/GSSG were both more oxidized (more positive) in females than in males. A 24-hour fast negated the sex differences in both redox potentials by oxidizing both redox couples in male mice, while having no effect on E_h Cys/CySS and a smaller effect on E_h GSH/GSSG in female mice. A diet with excess sulfur amino acids reduced the plasma E_h Cys/CySS in females to a level comparable to that seen in male mice. Thus, sex-specific differences in plasma E_h Cys/CySS could be normalized by two different dietary interventions. Some of these findings are consistent with what has been reported for human plasma redox potentials, while others are not. Most strikingly, mice do not exhibit the same age-dependent oxidation of the plasma redox potentials studied. Also, whereas both species have sex differences in E_h Cys/CySS, female humans are more reduced, while female mice are more oxidized than their male counterparts. Care must be taken when designing and interpreting mouse studies to investigate redox regulation in humans.

2543 Metabolome-Wide Association Study (MWAS) of Plasma Cystine in Healthy Aging Women

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Cystine (CySS) is an oxidized form of cysteine, abundant in plasma and having different distribution in males and females. Plasma CySS is a measure of oxidative stress and increases with age, but the mechanistic basis is unknown. We hypothesized that if environmental exposures causing oxidative stress in women contribute to increased CySS concentrations with age, a metabolome-wide association study of age should reveal environmental chemical associations with CySS. Aim: Perform metabolome-wide association study (MWAS) of plasma CySS in healthy aging women to test for association of environmental chemicals with CySS. Plasma from 65 women (20 to 62 y; mean, 42.6 y) and without known disease (PRE-MED cohort; NCT00336570) was used. Plasma CySS was quantified by HPLC (mean, 68.9 μM). High-resolution metabolomics of plasma was used for relative quantification of environmental chemicals and metabolites. Samples were analyzed by LC-HRMS with +ESI on C18; data were extracted by apLCMS and xMSanalyzer and log-transformed prior to biostatistics and bioinformatics. Metabolome correlations with CySS and age were performed by xMWAS using Spearman correlation ($r > 0.25$, $P < 0.05$). xMSannotator with HMDB and KEGG was used for metabolome annotation and with the Toxin and Toxin-Target DataBase (T3DB) for environmental chemical annotation. The results showed 265 metabolites positively associated with CySS and 285 positively associated with age ($r > 0.25$, $P < 0.05$). Of those associated with CySS, 30 matched environmental chemicals. A relatively high correlation with CySS was observed for phenol compounds (chloro-, nitro-, dinitrophenols) and lactofen ($r > 0.4$). Of metabolites associated with age, 23 matched environmental chemicals. Correlation of benzofuran compounds with age was relatively high ($r > 0.3$). The results show that environmental chemicals associate with plasma CySS in healthy aging women. The results indicate that cumulative environmental exposures or decline in elimination of environmental chemicals could contribute to increased oxidative stress with age.

2544 Rapid and Sensitive Mass Spectrometry Method for Simultaneous Quantification of Intracellular Reduced and Oxidized Glutathione

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Reduced glutathione (GSH) plays a key role in the protection of cellular components from damage caused by reactive oxygen species. The ratio of GSH to oxidized glutathione (GSSG) is an important marker for oxidative stress. It is a challenge to quantify precisely the concentrations of GSH and GSSG in biological samples because of the artificial oxidation of GSH during sample preparation and the low levels of GSSG. We have developed a sensitive and rapid mass spectrometry method for the simultaneous determination of GSH and GSSG in cultured cells. Free GSH was stabilized by the S-carbamidomethylation with iodoacetamide during sample processing. GSH and GSSG were analyzed using a Waters UPLC System coupled with a Waters ACQUITY QDa mass detector in the positive ion mode (ESI+) with a run time of 3 minutes. The limit of detections for GSH and GSSG were 4.66 and 1.14 ng/ml, respectively. The LC-MS method was validated following FDA Reviewer Guidance Validation of Chromatographic Methods. Linear calibration curves were obtained ranging from 0.625 μg/ml to 40 μg/ml with a R^2 of 0.999 for GSH and 1.25 ng/ml to 40 ng/ml with a R^2 of 0.998 for GSSG. This rapid and sensitive method has been applied successfully to measure the glutathione redox status in human hepatoma HepG2 cells, undifferentiated normal human primary bronchial epithelial cells and a differentiated human air-liquid-interface airway tissue model.

2545 The Long Noncoding RNA MALAT1 Regulates Oxidative Stress: Mechanism and Therapeutic Application for Multiple Diseases

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The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA and its overexpression is associated with the development of many types of malignancy. It is an evolutionarily highly conserved RNA with high level of expression in various tissues and cell types. MALAT1 null mice show no overt phenotype under normal laboratory conditions. However, in transcriptome analysis of MALAT1 null mice we found significant upregulation of nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) regulated antioxidant genes including Nqo1 and Cat with significant reduction in reactive oxygen species (ROS) in hepatocyte and pancreas. We performed lncRNA pulldown assay using biotinylated antisense oligonucleotides against MALAT1 and found MALAT1 interacted with Nrf2, suggesting Nrf2 is transcriptionally regulated by MALAT1. Exposure to excessive ROS has been shown to cause insulin resistance through activation of c-Jun N-terminal kinase (JNK) which leads to inhibition of insulin receptor substrate 1 (IRS-1) and insulin-induced phosphorylation of serine/threonine kinase Akt. We found MALAT1 ablation suppressed JNK activity with concomitant insulin-induced activation of IRS-1 and phosphorylation of Akt suggesting MALAT1 regulated insulin responses. MALAT1 null mice exhibited sensitized insulin-signaling response to glucose/insulin challenges and significantly increased insulin secretion in isolated MALAT1 null islets, suggesting an increased insulin sensitivity. Furthermore, we established two mutually supporting mouse T2DM model, high fat diet (HFD) and *ob/ob* model and investigated the role of MALAT1 in regulating the insulin signaling pathway and pancreatic β cell function in these two T2DM model. Consistent with our previous results, MALAT1 ablation alleviate the obesity-induced T2DM in *ob/ob* mice. With reduced ROS, MALAT1 null mice showed reduced lipopolysaccharide (LPS)-induced cytokines, suppressed NF-kB and NLRP3 inflammasome signaling pathway, alleviated inflammatory response, and in fact, MALAT1 null mice became resistant to septic shock in the LPS and cecum ligated punctuation (CLP)-induced septic shock mouse models. Our results showed that MALAT1 plays a pivotal role in the crosstalk between Nrf2-regulated detoxification pathway and the oxidative stress related diseases including T2D and proinflammatory response, suggesting ROS homeostasis is a point of convergence of the interactions between MALAT1-Nrf2 axis, and this axis could be a therapeutic target for treating insulin resistance, glucose dysregulation, LPS-induced inflammation and others diseases caused by excessive exposure to ROS.

2546 Differential Temporal Effects of Hyperoxia and Vitamin A on Gene Expression Profiling in Lungs of Newborn Rats: Implications for Bronchopulmonary Dysplasia (BPD)

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Supplemental oxygen is administered to premature infants having pulmonary insufficiency. However, hyperoxia contributes to the development of BPD in these infants. The molecular mechanisms of abnormal lung development observed in BPD patients are not completely understood. Vitamin A is a promising treatment regimen that might mitigate the negative long-term effects of hyperoxia exposure. In this study, we tested the hypothesis that exposure of newborn rats to hyperoxia for 7 days, followed by normoxia for another 15 days, will lead to differential temporal alterations in gene expression in pathways that are relevant to oxidative stress and abnormal lung development. We also assessed the effect of Vitamin A treatment in this phenomenon. Newborn Fisher 344 rats were exposed to hyperoxia (95%) or room air from postnatal day (PND) 1 to 8, and animals were either sacrificed on PND 8, or were returned to normoxia and were sacrificed at PND 22. Within each rearing condition, half of the pups were assigned to receive daily intraperitoneal (IP) injections from P1-P5 of either 2 mg/kg vitamin A dissolved in corn-oil, or vehicle (corn-oil, 33 mL/kg) alone. Total RNA was isolated from individual animals (n=3), and gene expression profiling was studied using RNA-seq on the Illumina HiSeq 2500 platform. Gene expression data was quantile normalized and analyzed using the DESeq2 software; significance was achieved for $q < 0.2$ and linear fold change exceeding 1.25x. We identified genes that significantly changed and in opposite direction on PND 22, comparing hyperoxia to normoxia group, and hyperoxia plus vitamin A compared to the hyperoxia group, and then determined enriched pathways (hypergeometric test, $q < 0.2$). Several pathways were altered, which included, neuronal, oxidative stress, and cytokine pathways. In addition, angiogenesis and vasculature development pathways were induced by hyperoxia at both PND 7 and PND 22, and suppressed by administration of vitamin A at PND 7. Therefore, we hypothesize that these genes could be potential targets for intervention in BPD patients.

2547 Cerebrovascular and Neurological Impact of Chronic Smoking on Post-Traumatic Brain Injury Outcome and Recovery: An *In Vivo* Study

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Traumatic Brain Injury (TBI) is one of the most common causes of cerebrovascular and neurological damage worldwide. To this end tobacco smoking (TS, one of the most addictive habit and a main public health hazards) affects the vascular inflammatory and neurovascular behavioral responses of the endothelium through oxidative stress (OS) stimuli enhancing the risk of various cerebrovascular and neurological disorders. It has been recently suggested that premorbid conditions such as TS leads to exacerbation of TBI and retardation of post TBI recovery. The aim of the present study is to investigate and dissect out the pathophysiological mechanisms underlying the exacerbation of TBI (simulated using a head weight drop model) following chronic TS exposure. For this purpose, male C57BL/6J mice, age range 6-8 weeks were exposed to TS for three weeks. Then, test animals were subjected TBI by head weight drop so that a metal weight (30 g) was inserted into a pre-positioned vertical guide whereas the weight free fell for a distance of 80 cm before reaching the target. Physical activity and weight of the mice were analyzed before, 1 hr, 24 hr and three days after TBI. Finally, mice were sacrificed to collect blood and brains samples for subsequent biochemical and molecular analysis. Western blotting was used to assess the expression of Nrf2 (a key antioxidant transcription factor) as well as tight junction proteins associated with BBB integrity including, ZO-1, Occludin, Claudin-5 from homogenized brain tissues. Levels of NF-kB (a pro-inflammatory transcript factor which antagonizes Nrf2 activity) along with pro-inflammatory cytokines IL-6, IL-10 and TNF-α were measured by ELISA on blood samples. The results revealed that TS promoted significantly increased inflammation and loss of BBB integrity in TBI when compared to TS-Free TBI mice. These were paralleled by significant worsening of weight loss, behavioral, and motor activity and an overall slower post-TBI recovery when compared to TF-free TBI mice. In conclusion TS leads to TBI exacerbation and retardation of pathophysiological recovery after TBI likely promoted by worsening of BBB impairment, and pro-inflammatory vascular activity induced by chronic smoking.

2548 Live Cell Imaging of Oxidative Stress in Human Airway Epithelial Cells Exposed to a Secondary Organic Aerosol

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Isoprene hydroxy hydroperoxide (ISOPOOH) is a major component of secondary organic aerosol (SOA) generated from the reaction of isoprene with atmospheric OH·, a reaction favored as pollution controls decrease levels of NO_x in the atmosphere. Relatively little is known about the contribution of ISOPOOH to the health effects from exposure to air pollutants. Previous studies have shown that ISOPOOH-generated SOA can induce oxidative stress in cultured human airway epithelial cells (HAEC). The objective of this study was to characterize the mechanisms of oxidative stress induced by exposure of HAEC to non-cytotoxic concentrations of ISOPOOH. Our experimental approach relies on live cell imaging of HBECs expressing the genetically encoded fluorogenic sensor roGFP, which reports specifically on changes in the glutathione redox potential (E_{GSH}) in real time. Low micromolar concentrations of ISOPOOH induced glutathione oxidation in HAEC through a mechanism that is independent of the generation of extracellular H_2O_2 but is effectively blocked by overexpression of catalase in HAEC, suggesting intracellular conversion of ISOPOOH to H_2O_2. The effect of ISOPOOH on E_{GSH} is potentiated by supplementation of the cells with selenium, consistent with the role of glutathione peroxidases in transducing the peroxidative tone presented by ISOPOOH to the glutathione pool. Similarly, deprivation of glucose sensitizes the cells to ISOPOOH-induced increases in E_{GSH}, suggesting the involvement of NADPH-dependent glutathione reductase activity in opposing ISOPOOH oxidative stress. These findings show that ISOPOOH is a potent environmental oxidant that likely contributes to the oxidative burden posed by inhalation of SOA. *This abstract does not necessarily reflect US EPA policy.*

2549 Prenatal Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) Augments Neonatal Hyperoxic Lung Injury and Alveolar Simplification and Causes Alterations in the Composition of Gut Microbiome in Mice: Mechanistic Role of Cytochrome P450 (CYP)1A1, 1A2, and 1B1

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Pregnant women living in the vicinity of superfund sites and smokers are at higher risk for preterm delivery, in part due to exposure to polycyclic aromatic hydrocarbons (PAHs). Preterm infants often require treatment with supplemental oxygen (hyperoxia) that in turn leads to a chronic lung disease of prematurity called bronchopulmonary dysplasia (BPD). Cytochrome P450 (CYP) enzymes have been implicated in this type of injury. CYP1A1 induction is protective against hyperoxic lung injury while CYP1B1 acts as a pro-oxidant. PAH administration augmented neonatal hyperoxic lung injury in a dose-dependent manner. The central hypothesis of this project is that prenatal administration of PAHs differentially exacerbates lung injury and causes gut dysbiosis in neonatal mice following postnatal hyperoxia, and that this effect is altered in mice lacking the gene for (Cyp)1a1, 1a2, or 1b1. Timed pregnant WT (C57BL/6J), Cyp1a1-null, Cyp1a2-null and Cyp1b1-null mice were treated orally with the vehicle corn oil (CO) or mixture of PAHs BP and BbF (7.5 mg/kg, each) on gestational days 16-19. The newborn mice obtained from these mothers were exposed to hyperoxia or room air for 14 days. On PND 14, the mice were euthanized, and lung injury was assessed by quantification of the radial alveolar count (RAC). Results showed that PAH exposure differentially exacerbates lung injury in WT, Cyp1a1-null, Cyp1a2-null and Cyp1b1-null mice and that hyperoxia caused reduction in RAC across all genotypes. PAH treatment resulted in significant induction of Cyp1a1 gene expression in room air, with suppression of Cyp1a1 expression following hyperoxia. In room air, Cyp1a1-null and Cyp1b1-null mice expressed significantly more TNF compared with WT mice. In hyperoxia, TNF and IL-6 expression were enhanced in all genotypes. 16S rRNA sequencing of gut microbiome samples at the PND14 timepoint revealed difference in Bray-Curtis beta diversity observed between PAH and CO groups in WT mice, and this was not seen in Cyp1a1-null mice, suggesting that Cyp1a-mediated metabolism of PAH plays a role in altering the intestinal microbiome. Future studies could lead to the development of novel strategies against BPD in premature infants exposed maternally to PAHs.

2550 Metabolic Adaptation in Macrophages as Mechanism of Defense against Crystalline Silica Dust

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Inhalation of respirable silica leads to silicosis, one of the most common occupational pneumoconiosis worldwide, associated with autoimmune diseases, lung cancer and increased risk of active tuberculosis by up to four-fold. About 2 million workers are at risk in the United States, but no specific therapy is available. Here, we hypothesize that macrophages exposed to silica undergo metabolic reprogramming, characterized by increased glycolysis, impaired Krebs cycle, suppressed mitochondrial oxidative phosphorylation, which leads to a pro-inflammatory phenotype. To test this hypothesis, murine macrophages RAW264.7 were exposed to silica (50 µg/cm2) with or without priming, with lipopolysaccharide (LPS, 1 ng/ml) and metabolic parameters were assessed after silica exposure up to 24 hours. To assess the effect of silica with and without LPS on mitochondrial respiration and changes in central carbon metabolism, we utilized state of the art instrumentation including the Oroboros O2k high-resolution respirometer and liquid chromatography-high resolution mass spectrometry (LC-HRMS). Lactate and lactate dehydrogenase (LDH) released in the supernatant was measured with lactate and LDH assay. ELISA assay was performed to measure pro-inflammatory cytokines, such as interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha, Western Blot assay to evaluate the hypoxia-inducible factor (HIF)-1α and Caspase 1, RT-PCR analysis to evaluate the gene expression of IL-1beta and TNF alpha. Exposure of cells to a non-toxic concentration of silica augmented the activation of complex II of the electron transport chain, independently from the complex I activity, to a greater extent than LPS-primed cells. In fact, stimulation of silica-exposed cells with succinate (CII activator), with or without pre-stimulation with rotenone (CI inhibitor), increased oxygen flux and reactive oxygen species (H_2O_2) more than LPS-exposed cells. As a result, HIF-1α stabilization, caspase-1 activation, and IL-1β release were significantly elevated in silica-exposed cells. However, LPS primed cells exhibited a higher transcription and release of TNF alpha. Secreted lactate was similar in silica- and LPS-exposed cells; while, the co-exposure increased lactate secretion, LDH, and cell death.

In conclusion, unlike the current opinion, silica alone can induce metabolic changes in RAW macrophages that eventually determine chronic inflammation and silicosis.

2551 Lung Fibroblasts from Idiopathic Pulmonary Fibrosis Patients Produce an Oxidizing Extracellular Redox Potential

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Idiopathic pulmonary fibrosis (IPF) is associated with increased deposition of extracellular matrix proteins, and lung fibroblasts are responsible for producing these pro-fibrotic proteins. One of the signals that evokes pro-fibrotic gene expression is a shift in the extracellular cysteine/cystine redox potential (E_h(Cys/CySS)) toward more positive (more oxidizing) potentials. Recently, we found that mouse lung fibroblasts can regulate their own extracellular E_h(Cys/CySS) and that changes in the activity of the CySS transporter Slc7a11 can change the value at which E_h(Cys/CySS) is maintained. Our hypothesis is that human lung fibroblasts from patients with IPF express low levels of SLC7A11 and produce an oxidizing extracellular E_h(Cys/CySS). IPF fibroblasts were isolated from explants of 6 IPF patients receiving lung transplant. Non-IPF fibroblasts were isolated from relatively normal lung tissues adjacent to cancerous tissues of 6 lung cancer patients. SLC7A11 mRNA levels were measured by qPCR. Concentrations of Cys, CySS, glutathione (GSH), glutathione disulfide (GSSG) and mixed disulfide (CySSG) were measured by HPLC. Nernst equation was used to determine the redox potentials. The data showed that extracellular E_h(Cys/CySS) was more oxidized for lung fibroblasts from IPF patients than those from non-IPF donors. Oxidation of E_h(Cys/CySS) in IPF fibroblasts was not due to decreased expression of SLC7A11. However, higher SLC7A11 expression was correlated with more reduced extracellular E_h(Cys/CySS) and more reduced intracellular E_h(GSH/GSSG) in non-IPF fibroblasts, but not in IPF fibroblasts. Therefore, SLC7A11-dependent regulation of extracellular and intracellular redox environments is lost in lung fibroblasts from IPF patients, suggesting that SLC7A11-independent mechanisms are responsible for aberrant redox regulation in this important effector cell of pulmonary fibrosis.

2552 Inhalation Co-exposure to Ultrafine Carbon and Ozone Leads to Significant Pulmonary and Systemic Oxidative Stress

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Particles and gases are integral components of ambient air pollution and both contribute significantly in adverse health outcomes. Recent epidemiologic data indicates that possible synergistic interactions between these components mediate the adverse phenotypes. Mechanistically, gaseous components can modify the particle surfaces and can reach distal portions of the lung, owing to the deeper penetrability of ultrafine particles. We hypothesize that ultrafine particles of carbon black (CB) and ozone (O_3) co-exposure will lead to significantly greater oxidative stress response in the lungs, heart and liver compared with individual/single toxicant exposures. We performed rodent (C57Bl/6J mice) whole-body inhalation exposures (air, CB, O_3 or CB+O_3) and studied acellular and cellular bimolecular free radical production by immuno-spin trapping. Mice were exposed to 2.0±0.02 ppm O_3 and/or 10±0.6 mg/m^3 CB for 3 hours (16 µg particle deposited dose). Aerosol mobility (140±2 nm), and aerodynamic (84±1 nm) diameters were measured by scanning mobility particle analyzer (SMPS 3938) and an electrical low-pressure impactor (ELPI+). Fourier transformed infrared (FTIR) spectroscopy demonstrated significant alteration in particle surface functional group composition after interaction with O_3. Ferric reducing ability of serum (FRAS) assay demonstrated significantly greater acellular oxidative potentials for co-exposure aerosol. A significantly greater increase in pulmonary and distal organ free radical production, xanthine oxidase activity and gene expression occurred after co-exposures compared with single exposures. In conclusion, our studies confirm interaction of gaseous and particle components of air pollution. By demonstrating distal organ oxidative stress response after pulmonary exposure, we describe a potential pathophysiologic mechanism for cardiovascular and hepatic dysfunction by air pollution exposure. Further mechanistic studies are underway to elaborate these findings using organoids and disease animal models. *Funding: NIH/NIGMS U54GM104942-03 (SH), NIH R01ES015022 (TRN), AHA 19TPA34850089, NIH HL 136383 (EK).*

2553 Glucocorticoids Regulate *SLC7A11* Expression and Sensitivity to Oxidative Stress

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SLC7A11 encodes an antiporter that exchanges glutamate for cystine. Cystine is a precursor to the antioxidant glutathione, and therefore, SLC7A11 activity increases cellular antioxidant capacity. *SLC7A11* is regulated by multiple stress responsive pathways, with altered expression implicated in multiple diseases. Using publicly available data we examined potential regulatory regions and hypothesized that the transcription factor (TF) glucocorticoid receptor (GR) represses *SLC7A11* expression. To test this, we treated A549 cells with ± 3µM mifepristone (MIF), a GR antagonist, for 24 hrs, then treated cells with ± 100nM dexamethasone (DEX), a GR agonist, for an additional 24 hrs. qRT-PCR measured expression of *SLC7A11* relative to a housekeeping gene, and the fold change relative to vehicle control was calculated. Treatment with DEX significantly repressed *SLC7A11* expression (0.43 ± 0.013, p=0.0001) and this was blocked by pre-treatment with MIF (0.90 ± 0.05), suggesting that DEX-mediated repression of *SLC7A11* is specific to GR. We then tested whether cells exposed to DEX are more susceptible to oxidative stress. A549 cells were treated with 100nM DEX (or vehicle) for 24 hours and then exposed to a wide concentration range of an inducer of oxidative stress, tert-Butyl hydroperoxide (TBOOH), or vehicle, and assessed for viability. Cells treated with DEX were more susceptible to TBOOH (+DEX EC50: 81.1µM, 0 DEX EC50: 230.9µM). qRT-PCR and TBOOH cytotoxicity experiments were repeated in HELA cells and rat astrocytes and the trends were consistent. Finally, we explored the potential *cis*-regulatory regions we identified at the *SLC7A11* locus using luciferase reporter assays; A549 cells were transfected with reporter constructs containing either the *SLC7A11* promoter, or the *SLC7A11* promoter plus *cis*-regulatory regions in intron 9 (I9) or intron 2 (I2). Following transfection, cells were treated with MIF and/or DEX in an exposure paradigm as described above. Repression was primarily mediated by the *cis*-regulatory regions in the promoter and I2. Further, we think that the repression in these regions is a result of GR tethering to another stress responsive TF, NRF2, and binding to an antioxidant response element. In summary: GR represses *SLC7A11* via *cis*-regulatory regions in the promoter and I2, likely via tethering to the TF NRF2. Repression of *SLC7A11* results in less exchange of glutamate for cystine, and this renders cells more susceptible to oxidative stress.

2554 Stat3 as a Novel Methylmercury-Induced Antioxidant Mechanism

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Methylmercury (MeHg) is an environmental pollutant that affects the developing and the mature central nervous system. Despite extensive research over the past decades, the molecular mechanisms mediating MeHg neurotoxicity have not been fully elucidated. Oxidative stress, impairment of the antioxidant defense and disruption of the calcium homeostasis are some of the mechanism associated with MeHg toxicity. The induction of nuclear factor erythroid 2-related factor 2 (Nrf2) and its role activating antioxidant response under MeHg-induced oxidative injury focus the attention of the scientific community as a target to counteract MeHg toxicity. Recent studies show that the Nrf2 signaling pathway is insufficient to prevent MeHg damage but there may be other protective mechanisms. The signal transducer and activator of transcription 3 (STAT3) has a pivotal role in cell growth and survival. Some studies also showed that STAT3 plays a role controlling redox homeostasis, preventing oxidative stress by a mechanism that includes modulation of nuclear genes encoding for electron transport complexes (ETC) and antioxidant enzymes. STAT3 also acts by non-classical mechanism, via mitochondria localization where interacts with complex I, increasing membrane potential and promoting ATP synthesis. All these features make STAT3 a plausible mechanism to prevent MeHg toxicity, in conjunction or as alternative to Nrf2 signaling. Here, using an immortalized neuronal hypothalamic GT1-7 cell line we tested if MeHg is able to induce the STAT3 signaling pathway. Our data show that MeHg exposure is associated with upregulation of STAT3-Y705 phosphorylation, inducing its nuclear translocation and regulating STAT3 associated gene expression. In addition, MeHg is also able to induce the phosphorylation at STAT3-S727, which is implicated in its mitochondrial localization. Moreover we found that the inhibition of STAT3 phosphorylation exacerbates MeHg toxicity increasing oxidative stress and mortality. Overall, we show that MeHg is able to induce both classical (nuclear) and non-classical (mitochon-

drial) STAT3 functions. The augmentation of MeHg toxicity by the inhibition of this pathway suggests that STAT3 participates in the antioxidant defense against MeHg injury.

2555 Sulfide Detoxification Is Prioritized over Butyrate Oxidation in the Human Colon

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Microbiota in the human gut metabolize fiber remnants from the diet to produce large quantities of butyrate, the primary energy source for colonic epithelial cells. In the mitochondria of these cells, short-chain acyl-CoA dehydrogenase (ACADS) is a flavoenzyme that catalyzes the initial step of butyrate oxidation, and relays the resulting electrons to the coenzyme Q_{10} pool. When ACADS is isolated from native or recombinant sources, a green color is observed in the protein that corresponds to its FAD cofactor bound to a coenzyme A persulfide (CoA-SSH). The CoA-SSH bound form of ACADS is inactive, and previous studies demonstrated that butyrate oxidation is impaired in colonic epithelial cells exposed to hydrogen sulfide (H_2S), suggesting that CoA-SSH may be generated under these conditions. H_2S, historically regarded as an environmental toxin, is produced at high concentrations by sulfate-reducing bacteria in the colon. However, toxic H_2S accumulation is prevented by sulfide quinone oxidoreductase (SQR), a mitochondrial membrane-bound flavoenzyme that oxidizes H_2S to a persulfide (R-SSH) with concomitant reduction of coenzyme Q_{10}. SQR is remarkably promiscuous in its use of acceptors to form persulfides, and was recently shown to contain an unusual active site cysteine trisulfide. We thus hypothesized that the source of CoA-SSH, and therefore ACADS inhibition, is H_2S oxidation via SQR activity utilizing CoA as an acceptor. In rapid kinetic studies, SQR facilitated sulfur transfer to coenzyme A, and steady-state kinetic assays further demonstrated that coenzyme A is a competent acceptor for the SQR reaction. Furthermore, SQR utilizing coenzyme A as an acceptor generates a charge transfer complex in ACADS corresponding to the CoA-SSH bound species, indicating that CoA-SSH is the persulfide product of the SQR reaction. As both ACADS and SQR relay electrons to coenzyme Q_{10}, competition over this substrate would hinder the effective clearance of toxic levels of sulfide. These studies implicate the inhibition of ACADS by CoA-SSH as a potential modulatory mechanism to reserve the coenzyme Q_{10} pool for SQR and prioritize sulfide detoxification. This would ensure that energy metabolism in the colon is not jeopardized during acute H_2S exposures.

2556 Peroxynitrite ($\pm CO_2$)-Mediated Oxidation of Dimedone Exemplifies Aliphatic Nitration: Significance to Nitration in Nitric Oxide-Producing Biological Systems

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Nitric oxide ($^{\bullet}NO$) is a versatile hormone apart from being a toxin; $^{\bullet}NO$ manifests its toxicity through the formation of peroxynitrite (PN) and the subsequent reaction of PN with endogenous CO_2 resulting in the formation of nitrogen dioxide ($^{\bullet}NO_2$) and the carbonate radical ($CO_3^{\bullet-}$). Numerous studies from our laboratory and elsewhere have shown that PN ($\pm CO_2$) brings about the oxidation of a vast array of biological molecules including the nitration of free and protein-bound L-tyrosine (Tyr). The reaction of Tyr with PN-derived oxidants is often considered a hallmark of electrophilic aromatic nitration. Using ethyl acetoacetate (EAA) as a model compound, we have previously demonstrated a case for aliphatic nitration by PN ($\pm CO_2$). Now, after two decades of the original work on EAA, we were surprised to learn that there have been no other reported examples of aliphatic nitration by PN ($\pm CO_2$), especially those employing enolizable cyclic ketones. In view this and also in view of a growing number of reports of PN reaction with membrane lipids forming signaling nitro products, we have examined the reactions of 5-dimethyl-1,3-cyclohexanedione (dimedone) by PN at pH of 7.2. It was found that dimedone reacts in this system to form a purple colored product that has a broad absorption centered around 548 nm. The yield of this product was markedly enhanced (*ca.* 20-fold) in the presence of physiologically relevant concentrations of CO_2, meaning that the product(s) is a nitro derivative formed mainly from the reactions of $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ with enolate form of dimedone. In the presence of CO_2, the yield of PN-mediated nitration increased with increase in the concentration dimedone and reached a plateau at concentrations above 15 mM. With varying concentrations of PN, the yield of nitrodimedone increased linearly with increase in the concentration. Unlike free 3-nitro-L-Tyr, the nitrodimedone is moderately sensitive to changes in the pH going from below 4 to above 10. Given the fact that there are numerous examples of the existence of enol and keto esters [acyl CoAs, phospho(enol)-pyruvate, etc] in biological systems, a detailed understanding of aliphatic nitration of dimedone can have significant implications to the $^{\bullet}NO$-producing biological systems especially in terms of the formation of certain long-acting $^{\bullet}NO$-like compounds. The reactions of PN with glucose, fructose, and certain other sugars have all been shown to have $^{\bullet}NO$-like activity.

2556a Blood Brain Barrier Impairment in the Context of Tobacco Smoke and HIV-1 gp120 Co-exposure

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The rate of tobacco smoking is exceedingly high in HIV infected individuals (40-75%) when compared with the general population (15%). Furthermore, 50% of the HIV infected population exhibits neurological complications including NeuroAIDS. We hypothesize that the oxidative stress caused by tobacco smoking and HIV-1 envelope protein gp120 may have a contributory effect on the increasing burden of NeuroAIDS related morbidity and mortality in HIV infected individuals who smoke. Increase in oxidative stress has been associated with the initial upregulation and subsequent overwhelming of Nuclear factor (erythroid derived 2)-like 2 [NFE2L2 or Nrf2] which regulates the expression of antioxidant proteins. Oxidative stress is also known to cause Blood Brain Barrier (BBB) dysfunction by downregulation of tight junction (TJ) proteins. Thus, we seek to develop an understanding of the underlying mechanisms that may contribute to the BBB impairment in relation to Tobacco Smoke and HIV-1 gp120 co-exposure. We studied the effects of co-exposure of Tobacco Smoke Extract and HIV-1 gp120 on Primary Human Brain Microvascular Endothelial Cells (HBMECs) and Primary Human Astrocytes by measuring Oxidative Stress and subsequent Antioxidant response, cells were treated individually with HIV-1 gp120 and Tobacco Smoke extract. Gp120 dose for *in vitro* studies was selected based on a MTT cell viability study. We assessed barrier function by measuring transendothelial electrical resistance (TEER) and permeability. Total proteins were isolated from each treatment group and examined for tight junction proteins (Occludin, ZO-1) expression. Generation of ROS was detected by DCFDA assay. Immunocytochemistry was performed to further confirm check the expression of Nrf2. Co-exposure of Tobacco Smoke Extract and HIV-1 gp120 on HBMECs and Human Astrocytes showed decrease in - Cell Viability, Blood Brain Barrier Integrity and Antioxidant response and increase in oxidative stress compared to control and individual treatments. Decrease in TEER measurements and the parallel increase in permeability to FITC was observed. ZO-1 and Occludin expression levels were reduced in the co-exposed group. Activation of the Nrf2 with its reduced expression in a group with co-exposed treatment indicated oxidative damage. Co-exposure to tobacco smoke extract (TSE) and HIV-1 gp120 (gp120) further aggravated the BBB endothelium dysfunction thereby worsening cerebrovascular condition.

2557 Elucidation of the Mechanism Involved in the Induction of Chemokine CCL4 Expression by Methylmercury

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Methylmercury is a harmful heavy metal found widely in the environment and causes central nervous disorders because this compound can cross the blood brain barrier. We have previously shown that C-C motif chemokine ligand 4 (CCL4) protects cultured neural cells from methylmercury toxicity and expression of CCL4 is specifically induced in mouse brain by methylmercury. In addition, the expression of CCL4 was induced prior to methylmercury toxicity, and CCL4 had a protective effect against methylmercury toxicity in mouse neural stem cells. These findings suggest that the induction of CCL4 expression by methylmercury may be a protective response to methylmercury toxicity. Therefore, elucidating the mechanism involved in the induction of CCL4 expression by methylmercury is important for understanding the brain-specific toxicity exhibited by methylmercury. In this study, we examined the transcriptional regulatory mechanism that induces CCL4 expression by methylmercury using C17.2 mouse neural stem cells. The promoter region of the CCL4 gene was analyzed by a reporter assay, revealing that the region up to 50 bp upstream from the transcription start site was necessary for inducing expression of CCL4 by methylmercury. Nine transcription factors that might bind to this upstream region and be involved in the induction of CCL4 expression by methylmercury were selected, and the induction of CCL4 expression by methylmercury was suppressed by the knockdown of serum response factor (SRF). In addition, the nuclear level of SRF was elevated by methylmercury, and an increase in the amount bound to the CCL4 gene promoter was also observed.

Furthermore, we examined the upstream signaling pathway involved in the induction of CCL4 expression by SRF, and confirmed that activation of p38 and ERK, which are part of the MAPK pathway, are involved. These results suggest that methylmercury induces the expression of CCL4 by activating SRF via the p38 and ERK signaling pathway. Our findings are important for elucidating the mechanism involved in the brain-specific induction of CCL4 expression by methylmercury.

2558 Evaluation Total and Speciated Urinary Arsenic and Risk of Acute Hepatitis E in the US Population

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Arsenic is a known toxic chemical and has immunomodulatory properties. Arsenic has the ability to change the susceptibility of infection in humans. Acute hepatitis E infection is an infectious disease; it can be self-limiting and in severe cases, can cause acute-on-chronic liver failure. The presence of Hepatitis E IgG (Immunoglobulin G) Antibody (IgG Anti-HEV) in blood represents acute hepatitis E infection in an individual. Very few studies have investigated the association between exposure to arsenic and acute hepatitis E infection risk in humans. The primary objective of this study was to assess the relationship between total urinary arsenic and speciated urinary arsenic (Urinary Arsenous Acid, Urinary Arsenic Acid, Urinary Arsenobetaine, Urinary Arsenocholine, Urinary Dimethylarsinic Acid, Urinary Monomethylarsonic Acid) and the presence of Hepatitis E IgG Antibody (IgG Anti-HEV). The 2011-201, 2013-2014, and 2015-2016 National Health and Nutrition Examination Survey (NHANES) III data set were analyzed, and participants were aged 20 years and older (n=7,061). We used weighted logistic regression to calculate Odds Ratios (OR) and Confidence Interval (CI) of the relationship between total and speciated urinary Arsenic concentrations and IgG Anti-HEV. For each analyte considered, a separate weighted logistic regression model was fitted. Each of these models regressed log-transformed analyte levels on the log-odds of the presence of IgG Anti-HEV. All pairwise Pearson correlation coefficients were computed for each of the urinary arsenic measurements. Of the included human subjects, 6,628 (93.9%) were negative for Hepatitis E IgG Antibody (IgG Anti-HEV) while 433 (6.1%) had a positive test for IgG Anti-HEV. Total urinary arsenic (OR= 1.16, CI= 1.04-1.30, p-value= 0.01), Urinary Arsenobetanine (OR= 1.17, CI= 1.08-1.27, p-value= 0.0006), and Urinary Dimethylarsinic (OR= 1.18, CI= 1.01-1.39, p-value= 0.04) were observed to be significantly associated with the increased risk of IgG anti-HEV. Elevated urinary arsenic was associated with the risk of acute hepatitis E infections in this study. Further research is needed to confirm the causal relationship between arsenic exposure and acute hepatitis E infections.

2559 Heavy Metals Concentrations and Physiochemical Characteristics of Underground Water Sources around the Open Dump Site, Igando, Lagos

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The rapid urbanization and industrialization in the World causes Heavy metals contamination in water, which is one of the major quality issues in many developing Countries. Heavy metals in the earthly environment pose a serious risk to human health. This study measured the heavy metals concentration and physiological characteristics of underground water sources around the open dump site in Igando, Lagos Nigeria. 12 different locations of Underground water samples from dug wells and boreholes within a distance range of 0.07-0.72 km form the dump site was collected. Structured questionnaire was used to determine the perception of the residents around the dump site about ill health effect of heavy metal contaminated water on humans, were administered to 10 users of each water sources making a total of 120 respondents. The selected relevant physicochemical parameters (colour, pH, electric conductivity, total hardness, nitrate and phosphate) and heavy metals (copper, zinc, iron, lead, manganese, nickel and cadmium) in the samples were analysed using commercial kit and Flame Atomic Absorption Spectrometer. Investigation revealed that the farther away the underground water from the dump site the lower the physiochemical parameters. The results show that there is a high concentration of the heavy metals in the various underground water samples with lead, Cadmium, Iron and Nickel having mean concentrations above the permissible limits specified by World Health Organization (W.H.O) and Standard Organization of Nigeria (S.O.N) for drinking water. This study suggests that the underground water neither near nor far from the dump site is polluted with heavy metals and respondents using this water are ignorant of the risk to health.

2560 Genetic Inhibition of Liver Manganese Import Corrects Aberrant Liver Erythropoietin Production in a Mouse Model of Inherited Manganese Excess Due to Deficiency in the Manganese Transporter *Slc30a10*

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Manganese (Mn) is a trace element essential for growth and development. Elevated levels of Mn can be toxic. Inherited diseases of Mn excess are caused by mutations in the Mn transport proteins SLC30A10 and SLC39A14. Though both are diseases of Mn overload, they do not showcase the same set of phenotypes: SLC30A10 mutations lead to high blood and tissue Mn levels, neurologic deficits, cirrhosis, and polycythemia whereas SLC39A14 deficiency results in high blood and tissue Mn levels (except liver) and neurologic deficits without cirrhosis or polycythemia. Patients with SLC30A10 mutations also have high serum levels of erythropoietin (Epo). These phenotypic differences led us to explore the unappreciated biological effects of Mn excess in the mammalian body. We developed a Slc30a10-deficient mouse model (*Slc30a10$^{KO/KO}$*), which recapitulated key human disease phenotypes including polycythemia and Mn accumulation in the blood and tissues (liver, bone, brain). Given that polycythemia is a unique manifestation of Mn toxicity, we aimed to understand how Mn overload results in polycythemia using *Slc30a10$^{KO/KO}$* mice as a tool. We hypothesized that Mn, like other divalent metals such as cobalt, acts as a 'hypoxia-mimetic' to upregulate expression of *Epo*, a hypoxia-regulated gene. Using RNA analyses, we identified liver as a key organ for aberrant Epo expression, rather than kidney, the physiological site of Epo expression. Expression profile of over 90 hypoxia-regulated genes using PCR arrays indicated upregulation of 24% genes in *Slc30a10$^{KO/KO}$* liver. Using gene-specific qPCR, we validated six genes (*Epo*, *Serpine1*, *Hk2*, *Anxa2*, *Lox*, and*Hif3a*) that showed highest differential expression. Next, we challenged our working model by genetically inhibiting liver Mn import by rendering mice deficient in Slc39a14, a Mn import protein responsible for liver Mn loading. Mice deficient in both Slc30a10and Slc39a14showed significantly lower liver Mn levels, liver *Epo* RNA levels, and red blood cell counts when compared to *Slc30a10$^{KO/KO}$* mice. RNA analyses also indicated a trend towards restoration of kidney *Epo* expression in *Slc30a10$^{KO/KO}$ Slc39a14$^{KO/KO}$* mice. Together, these studies suggest that liver Mn excess drives aberrant Epo expression in *Slc30a10$^{KO/KO}$* mice and add to our understanding of novel mechanisms of Mn toxicity.

2561 A Population Toxicokinetic Modeling of Mercury and Methylmercury in Human

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We have previously published about half-lives of whole blood and hair for methylmercury kinetics via tuna and swordfish intake (Yaginuma-Sakurai et al., J Toxicol Sci. 2012: 123-30). In this study, we aimed to carry out a detailed analysis of the population toxicokinetic modelling of methylmercury using data obtained from Yaginuma-Sakurai et al (2012). A bigeye tuna and swordfish equivalent to the tolerable weekly intake of methylmercury was ingested for 14 weeks, followed by an observation period of 15-28 weeks. Blood collection was performed each one week for 14 weeks, and every two weeks after 15-28 weeks (N = 27). The population toxicokinetics modelling of mercury and methylmercury was performed using plasma mercury concentration and plasma methylmercury concentration. As a basic model of population toxicokinetics, a proportional error model (log additive) of a one-compartment model with the best model fit was selected from information such as minimum AIC. As a result, the median of the calculated mercury elimination rate constant was 0.082, and the median half-life was calculated to be 59 days. The median value of methyl mercury elimination rate constant was 0.087, and the median half-life was calculated to be 56 days. The half-life of blood mercury shown in the previous study was 46 to 53 days, and almost equivalent results were obtained.

2562 Variation in Blood Lead Accumulation Is Strongly Influenced by Genetics and Diet

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Although physiological effects and public health concerns of the environmental heavy metal lead (Pb) are well documented, we know little about the genetic and/or dietary factors that determine individual variability in

susceptibility to lead poisoning. Traditional epidemiological approaches are limited in exploring factors contributing to differential susceptibility due to heterogeneity in genetic background and uncontrolled environments. These limitations are particularly problematic when using blood measurements of toxicants like Pb as a surrogate for exposure. To overcome these limitations, new genetically-diverse mouse resources such as the Collaborative Cross (CC) have been developed to better model the genetic diversity found in human populations under controlled environments. To specifically address the influence of genetic and dietary factors on Pb bioaccumulation, we utilized the CC to identify genetic polymorphisms that determine variation in Pb blood levels in the context of a common exposure level. Adult females from 50 different CC lines received either mouse chow or American diet - a dietary pattern characterized by high intake of sugar and fat - and Pb (0.1%) through drinking water *ad libitum*. CC lines showed significant variation in blood lead levels (BLLs) depending on genetic background, diet, and genetic background-by-diet interactions. Genetic association analysis identified haplotypes associated with variation in BLLs in response to diet. Distinct haplotypes correlated with elevated BLLs originated from the PWK strain, one of wild-derived founders of the CC. Using this model, we aim to elucidate mechanisms by which genetics and diet contribute to lead bioaccumulation, how these can be used to better evaluate exposure levels from indirect BLLs, and provide prevention strategies and dietary interventions to reduce adverse effects of lead exposure.

PS 2563 Chromium and Vanadium Have Gene-Environment Interactions in Shared Biological Pathways

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The unregulated contaminants vanadium and chromium often co-occur in public drinking water systems in the United States, raising concerns about possible hazards from joint exposure. On October 9, 2019, we queried the Comparative Toxicogenomics Database (CTD), a publicly available curated database cataloguing gene-environment interactions, to explore possible shared toxicological pathways affected by these exposures. There were 149 genes suggested by scientific literature indexed in CTD to have interactions with vanadium for *Homo sapiens*, and 704 genes showing interactions with chromium for *Homo sapiens*, with 16 genes appearing in both lists: *ARNT, ATP2B1, CDK17, CDK6, CXCL8, HIF1A, IL6, INSIG1, MAPK1, MAPK3, MMP1, PTK2B, SLCO30A4, TIMP1, TNF,* and *TP53*. We entered these 837 unique genes interacting with either metal into the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 as official gene symbols; 803 of the genes in this list were recognized with *Homo sapiens.* Using DAVID's Functional Annotation Tool allowed us to visualize affected toxicogenomic pathways from the KEGG PATHWAY database. The KEGG minimum threshold for genes present in enriched pathways was set to 2. The top hit was "Pathways in Cancer" with 84 pathway genes (P value 4.4 E-23, Benjamini P value 1.2 E-20). The next pathway hit was "Hepatitis B" with 42 pathway genes (P value 2.2 E-16, Benjamini P value 2.9 E-14). Some more specific, interesting pathways suggested were the "FoxO signaling pathway" with 39 pathway genes (P value 3.0 E-15, Benjamini P value 2.0 E-13), the "TNF signaling pathway" with 33 pathway genes (P value 1.0 E-13, Benjamini P value 4.5 E-12), the HIF-1 signaling pathway with 31 pathway genes (P value 1.7 E-13, Benjamini P value 5.6 E-12), and the PI3K-Akt signaling pathway with 57 pathway genes (P value 1.2 E-10, Benjamini P value 2.5 E-9). This hypothesis-generating analysis of publicly available toxicogenomic databases suggests several biological pathways that merit further investigation in joint-exposure drinking water experimental toxicology studies and in molecular epidemiology studies.

PS 2564 microRNA Expression Influences Methylmercury-Induced Mitochondrial Dysfunction

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Mitochondrial dysfunction is a hallmark of several conditions, including Parkinson's disease and metabolic disorders. Methylmercury (MeHg) causes mitochondrial dysfunction through inhibiting respiration and inducing oxidative stress, however molecular targets contributing to MeHg-induced mitochondrial dysfunction have not been fully described. MicroRNA (miRNA) are emerging regulators of mitochondrial function and are responsive to environmental agents. We hypothesized that down regulating miRNA expression in *Caenorhabditis elegans* would exacerbate MeHg-induced mitochondrial dysfunction by suppressing mitoprotective miRNA. Worms that express mutations in *rrf-1* or *rrf-3* are more sensitive to the effects of miRNA, while worms that express mutant *pash-1* or *nrde-2* are more resistant to miRNA than wild type (N2) worms. MeHg significantly decreased oxygen consumption in N2 worms, which was further decreased in the miRNA resistant strains. Oxygen consumption was significantly improved following MeHg treatment in miRNA sensitive strains as compared to N2. Concurrent with the decrease in respiratory function was depolarization of the mitochondrial membrane potential by MeHg. miRNA resistant strains had significantly more membrane depolarization than N2, whereas miRNA sensitive strains had less depolarization than N2. MeHg decreased the mitochondrial mass of the worms and decreased the total mitochondrial DNA (mtDNA) copy number in N2 worms. This suggests that MeHg not only decreases the number of mitochondria, but also impairs the ability to replenish mitochondria following insult. Loss of mtDNA copy number and mitochondrial mass were exacerbated in the miRNA resistant strains, and were milder in the miRNA sensitive strains. Taken together, our data support a role for mitoprotective miRNA expression in response to MeHg exposure. Inhibition of protective miRNAs induces further damage to the mitochondria by MeHg.

PS 2565 Particulate Hexavalent Chromium Induces Cytotoxicity and Genotoxicity in Female and Male Fin Whale Cells

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Hexavalent chromium is a global health concern, a known human carcinogen as well as reproductive and developmental toxicant. Although it is clear that DNA damage, manifested as strand breaks and chromosomal changes plays a central role, the mechanism underlying its carcinogenicity is uncertain. Chromium is a ubiquitous marine pollutant and high chromium levels have been reported in North Atlantic whales including fin whales. Whale species represent a good model for toxicological studies because despite the fact that marine mammals accumulate high amounts of pollutants due to their long lifespan and deep diving behavior, they are known to be less susceptible to develop cancer than humans. Studying the toxicity of chromium in whale cells might be key to acquire a better understanding of the underlying toxicity of chromium. Therefore, we hypothesized that acute (24 h) and prolonged (120 h) exposures of particulate Cr(VI) to fin whale male and female cells induces both cytotoxic and genotoxic effects. To investigate this, cytotoxicity was measured by the clonogenic assay also known as colony forming assay, which measures the ability of cells to proliferate and form colonies after the treatment. Clastogenicity was measured using the chromosome aberration assay. Intracellular chromium levels were also determined with Graphite Furnace Atomic Absorption Spectroscopy. As hypothesized, particulate Cr(VI) induced a cytotoxic and genotoxic response in a concentration dependent manner in cells from both sexes. Both acute and prolonged exposure induced similar amounts of cytotoxicity, but prolonged exposure induced less genotoxicity than acute exposure. Intracellular chromium was lower after prolonged exposure than acute exposure. Overall, compared to previous data in human cells, both fin whale sexes showed a resistance to Cr(VI), as prolonged particulate Cr(VI) exposures in human cells showed an increased amount of toxicity. This outcome suggests marine mammals may have developed cellular mechanisms to counterbalance chromium induced toxicity. In the future, assessing cellular mechanisms activated after the Cr(VI) exposure, such as, Cr transport, DNA repair and cell death pathways, could provide insights into why fin whale cells are relatively resistant to genotoxic agents. *Supported by NIEHS grant ES016893 (J.P.W.).*

PS 2566 Adverse Effect of Manganese Exposure on Adult Neurogenesis: Evidence from the Subventricular Zone (SVZ)-Derived Neurosphere Assay *In Vitro*

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Manganese (Mn) is an essential metal to human health; however, excessive exposure to Mn is known to cause clinical symptoms with motor and non-motor dysfunctions similar, but not identical, to Parkinson's disease (PD). Earlier studies from this lab have established a detrimental effect of subchronic Mn exposure on adult neurogenesis (ANG) in SVZ. This study was designed to test the hypothesis that Mn exposure selectively affected the proliferation, migration, and differentiation of neural stem/progenitor cells (NSPCs) derived from the adult SVZ. Neurospheres were generated by isolating SVZ tissues from young adult mice (6-10 weeks old); cells were cultured in a growth factor-enriched medium. The newly formed neurospheres with the size of ~ 200 µm were selected and seeded on poly-L-ornithine-coated coverslips. The cultures were then treated with or without Mn-containing medium at 0, 0.1, 1.0, or 10.0 µM for five days. The cell proliferation, migration, and differentiation were determined by measuring the distance of newly generated cells migrated from neurospheres, by BrdU (5-Bromo-2′-deoxyuridine) incorporation assays, and by quantifying DCX and NeuN immunofluorescence, respectively.

Our data showed that *in vitro* Mn exposure significantly inhibited cell migration in a dose-dependent manner. For example, compared to the control group (628.64 ± 147.41 μm), Mn treatment at 10 μM reduced the migration distance (417.1 ± 57.31 μm) by 33.6% ($p<0.05$). The data from immunofluorescence assays did not reveal a significant change in BrdU(+) proliferating cells following Mn treatment at all dose levels. Interestingly, however, cell counts of DCX(+) neuroblasts per neurosphere suggested a significant reduction in Mn-treated cells at 10 μM (83.00 ± 23.26), as compared to the control group (241.3 ± 78.68), a reduction of 65.6% ($p<0.05$). Moreover, NeuN(+) mature neurons in the Mn-treated group at 10 μM (107.0 ± 19.52) in neurospheres were reduced by 33.5% ($p<0.05$) as compared to controls (161.00 ± 21.66). Taken together, these findings indicate that Mn exposure selectively affected the different stages of the AGN in SVZ, particularly on migration and differentiation of newly formed cells. How this may influence Mn caused motor or non-motor dysfunctions remains unclear and thus deserves further investigation. Mechanistic investigation of key signaling pathways in Mn-induced aberrant SVZ neurogenesis is currently in progress.

2567 Identification of Methyltransferases for Production of a Urinary Selenometabolite, Trimethylselenonium

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Selenium (Se) is an essential element for animals, and mostly excreted into the urine. Within the nutritional level to the low toxicity level, the major urinary metabolite is *Se*-methylseleno-*N*-acetyl-galactosamine (MeSeGalNAc). The second major urinary selenometabolite is trimethylselenonium ion (TMSe), which is simply the methylated compound of selenide, a key metabolic intermediate. Two methyltransferases are speculated to involve in the Se methylation. Thiopurine *S*-methyltransferase (TPMT) is first identified to catalyze sulfur methylation of sulfhydryl group in 6-mercaptopurine. TPMT is also suggested to perform a Se methylation. The other is indolethylamine *N*-methyltransferase (INMT). In human, a genome-wide association study for the TMSe-producing and non-producing individuals revealed the correlation between single nucleotide polymorphisms in *INMT* gene and the concentration of TMSe in blood and urine. However, molecular mechanisms underlying the methylation from selenide to TMSe are still unclear. We revealed that these two methyltransferases cooperatively and substrate-specifically catalyzed the selenide methylation to form TMSe.

2568 Active Bacterial Copper-Efflux Response Contributes to Environmental Toxicity in *Caenorhabditis elegans*

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Environmentally ubiquitous transition metals challenge prokaryotic and eukaryotic species through uncontrolled redox cycling. When these ions are overly abundant, all extant organisms contain largely conserved mechanisms (chelation, sequestration or excretion) to minimize damage and increase chances for survival. However, organismal toxicity is not only dependent on absolute abundance, but also speciation, complexation, and other environmental factors that contribute to overall ion availability. For instance, lower concentrations of copper (Cu) are more toxic to *Caenorhabditis elegans* (*C. elegans*), a free-living nematode, when grown on a diet of dead *Escherichia coli* (*E. coli*), as opposed to *E. coli* that is alive. The cause of this discrepancy goes beyond passive metabolic functions because cadmium (Cd), another transition metal, is equally toxic to *C. elegans* regardless of the bacterial status. From these observations, we hypothesize that an active and specific bacterial response mediates Cu toxicity in higher organisms. To test this hypothesis, we took advantage of the *C. elegans*/*E. coli* model system to examine bacterial factors that contribute to the environmental toxicity of Cu. Using *E. coli* knockouts with 100%, 50% and 25% of wildtype Cu-efflux capacity, we discovered a significant relationship between this active bacterial response and the severity of metal toxicity in *C. elegans*. Comparing defined Cu-toxicity endpoints in *C. elegans* fed *E. coli* with wildtype Cu-efflux capacity (100%) to those fed *E. coli* with reduced efflux capacity (25 to 50%), we observed 1) reduced lethality from 8.5 days of median survival up to 14.5 days ($p > 0.0001$), 2) a 7.6% ($p=0.0001$) increase in length independent of dauer arrest, 3) a 10-fold ($p=0.0008$) reduction in cumulative risk for egg-laying defects comparable to no-exposure control risk levels, and 4) reduced transcription of a known *C. elegans* metal-response marker. Conversely, overall body burden of the metal in *C. elegans* appeared unaffected by bacterial Cu-efflux capacity and entirely dependent on $CuSO_4$ supplementation in growth media. Current results support the bacterial Cu-response as an important contributor to the severity of metal toxicity in higher organisms. Ongoing work seeks to better define the *C. elegans* transcriptional activity during these conditional Cu exposures.

2569 *In Vivo* Dosing with Cadmium Causes Decreased Glucose-Stimulated Insulin Secretion in Isolated Pancreatic Islets

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Diabetes mellitus (DM) is a growing worldwide epidemic. Decreased insulin secretion in the face of insulin resistance is the hallmark of type 2 DM. Epidemiological and experimental studies show that exposure to the metal cadmium (Cd) is associated with pre-DM, DM and altered plasma insulin. How Cd disrupts pancreatic islet function and glucose-stimulated insulin secretion is not known. The objective of the current study was to begin to characterize the *in vivo* toxic effects of Cd on pancreatic islets. To do this, we used a model of long-term Cd exposure by administering Cd in the form of $CdCl_2$ (0.6 mg/kg/day 5 days per week for up to 12 weeks) in male and female Sprague Dawley rats. Pancreatic islets were isolated from 6 and 9 week treated animals and allowed to recover overnight. Islets were then incubated in high (4.5 mg/ml) glucose-containing buffer for 6 hours. Samples of buffer were collected and islets lysed. Percent insulin release was quantified by measuring buffer insulin content normalized to islet lysate insulin. After 6 weeks of treatment, percent insulin release (mean ± SE) from saline-treated control female and male animals were 30.3 ± 9.2% and 38 ± 8.87%, respectively. Cadmium treatment resulted a decrease in percent insulin secretion with 23.3 ± 1.9% and 19.3 ± 5% for female and male animals, respectively. After 9 weeks of treatment, percent insulin secretion from female and male control-treated animals were 29.7 ± 7.8% and 45 ± 17.6%, respectively; with Cd treatment the average values were 22 ± 4.5% and 19.6 ± 11% for female and male animals, respectively. Two-way ANOVA indicated significant differences between control and Cd-treatment groups; however, post-hoc Tukey's tests did not indicate statistically significant changes. These data from 6 and 9 week treated animals show that Cd exposure results in significant reductions in glucose-stimulated insulin secretion from pancreatic islets. There was no apparent time-dependent response indicating that this effect is independent of increasing Cd accumulation within pancreatic islets that occurs over time. This study reinforces that Cd is a diabetogenic substance that acts by altering pancreatic islet function.

2570 The Role of Activator Protein 1 (AP-1) and Nuclear Protein 1 (Nupr1) in Nickel-Induced Carcinogenesis

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Nickel (Ni) is used in a variety of industries including stainless and alloy steel production, electroplating, ceramics, pigments, and as a chemical intermediate, primarily because of its desirable properties such as corrosion resistance, heat resistance, hardness, and strength. Ni is also a well-known human carcinogen based on its ability to cause cancers of the lung and nasal cavity, and several millions of people are occupationally and environmentally exposed to nickel worldwide. However, the mechanisms of Ni carcinogenicity remain elusive. Nupr1, a small stress protein, is involved in cell transformation and carcinogenesis. Nupr1 has been implicated in a number of cancers including lung, breast, and pancreatic cancers. In this study, we found using quantitative real-time PCR and Western blotting that Nupr1 expression was increased following Ni exposure at both the mRNA and protein levels in human bronchial epithelial cells (BEAS-2B) in a dose-dependent manner. Nupr1 was also upregulated in Ni-transformed cells, suggesting that induction of Nupr1 may play important roles in Ni-induced carcinogenesis. To investigate which regulatory regions within the Nupr1 promoter are responsible for transcriptional activation following Ni exposure, the Nupr1 promoter (-2500 to +500) was cloned and inserted into luciferase reporter constructs. Promoter bashing experiments determined a region that mediates Ni-induced Nupr1 transcriptional activation. Promoter activation by Ni in a stable transfection model is also being investigated. Computational prediction of transcription factor binding sites identified an AP-1 motif within the region identified. In fact, mRNA and protein expression of AP-1 subunits c-JUN, c-FOS, ATF-2, and FOSL1 were found to be increased in a dose-dependent manner following Ni exposure in BEAS-2B cells and in human embryonic kidney cells (HEK-293). Notably, knockdown of AP-1 (c-JUN and c-FOS) using RNA interference suppressed Ni-induced Nupr1 expression, indicating that Ni induces Nupr1 through upregulating AP-1 expression. In summary, this study demonstrates that Ni is capable of inducing AP-1, which regulates the expression of Nupr1 and may

contribute to Ni-mediated carcinogenesis. Understanding the mechanisms governing Ni-induced carcinogenesis may apply to additional environmental and occupational carcinogens, and provides valuable insight into the carcinogenic process in general, which may be useful for identifying potential chemotherapeutics.

2571 Proximal Tubular Hypertrophy Enhances Basolateral Uptake of Mercury

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Approximately 15% of the United States population has been diagnosed with chronic kidney disease (CKD). CKD is characterized by a progressive and permanent loss of functioning nephrons. Following this loss, the remaining functional nephrons undergo compensatory changes including increased renal blood flow, increased single nephron glomerular filtration rate (SNGFR), and cellular hypertrophy. We hypothesize that compensatory changes in proximal tubular cells leads to enhanced cellular uptake of mercury (Hg). Mercury is a ubiquitous environmental toxicant to which humans are exposed through various routes. Patients with CKD may be more susceptible to Hg and thus, it is important to understand how Hg is handled in kidneys of these patients. We hypothesize that compensatory hypertrophy of proximal tubules leads to an increase in the uptake and accumulation of Hg. Furthermore, we propose that hypertrophied tubules are more sensitive to the toxic effects of Hg. To test these hypotheses, we used *in vitro* and *in vivo* models. Basolateral uptake of Hg, as a conjugate of glutathione (GSH; GSH-Hg-GSH), was measured in isolated non-perfused proximal tubules from control and nephrectomized rabbits. The effects of Hg on biochemical aspects of oxidative stress were measured in kidneys from rats exposed to Hg. These findings show that hypertrophied tubules take up and accumulate more Hg than normal tubules. In addition, hypertrophied tubules appear to be more sensitive to the effects of Hg than normal tubules. These data provide important information regarding the handling of mercuric ions in patients with renal insufficiency.

2572 Toxicity Profile of a Novel Analogue of Carboplatin in Cellular Models of Cancer

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There are currently three FDA platinum compounds approved for use as chemotherapeutics, where each drug has variable efficacies for different cancer types depending on the tissue where the cancer originated. The approved compounds are platinum(II) complexes with four coordination sites on the platinum atom allowing two types of ligands to attach: leaving ligands, which are removed from the platinum atom in solution, and non-leaving ligands, which remain complexed to the platinum in solution. This study aims to examine the potency of a novel analogue and compare it to one of the approved drugs, carboplatin. Carboplatin, the preferred compound used to treat ovarian and small-cell lung cancers, has a characteristic cyclobutanedicarboxylate leaving ligand and two ammonia non-leaving ligands. Our novel compound, 1,1-cyclobutanedicarboxylato(ethylenediamine)platinum (II), or Pt(en)CBDCA, has an ethylenediamine group in place of the ammonia ligands. We hypothesize that the efficacies of platinum compounds vary due to differences in the leaving and non-leaving ligands. To understand the impact of this structural change, we are investigating the analogue's effects on cell viability by performing MTT assays in three distinct human cancer cell lines. Preliminary data shows a large difference between IC-50 values of the melanoma cells and the colorectal cells treated with both Carboplatin and Pt(en) CBDCA. Given that intracellular accumulation is typically correlated to toxicity, we hypothesize that structural differences may alter intracellular accumulation of the compounds. To test our hypothesis, we are examining intracellular levels of both carboplatin and Pt(en)CBDCA using atomic absorption spectroscopy. In this study, we aim to correlate the intracellular concentration of platinum with cell survival response to create a toxicity profile of these compounds.

2573 Investigating a Role for Gut Microbes in Modulating the Toxicokinetics of Methylmercury

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Methylmercury (MeHg) poses a risk to human health due to its widespread prevalence and its potential as a developmental neurotoxicant, capable of causing a range of neurocognitive and neuromotor deficits, particularly after *in utero* exposure. Human MeHg exposure is primarily through the consumption of predatory fish. With fish consumption rising globally, exposure to MeHg is inevitably difficult to regulate. To protect the developing fetus, regulatory agencies have established a reference dose for MeHg, creating a "one size fits all" standard for fish consumption. The inability to eliminate MeHg from the human diet, and the "one size fits all" regulatory approach, underscores a need to investigate inter-individual differences in MeHg toxicokinetics and susceptibility. Prior studies, and evidence from our lab analyzing MeHg elimination in individuals consuming fish, demonstrate high variability in their MeHg half-lives ($t_{1/2}$), ranging from 30 to >120 days. It has also been shown, in both rodents and humans, that antibiotic exposure increases the $t_{1/2}$ of MeHg. These findings lead us to hypothesize a role for the gut microbiome in impacting the kinetics of MeHg metabolism and excretion. Specifically, we hypothesize the presence of gut bacterial species capable of demethylating MeHg to produce inorganic Hg, a form more rapidly eliminated. Bacterial genes identified within the mer operon, specifically, organomercurial lyase (MerB) and mercury reductase (MerA), are capable of MeHg demethylation and reduction to Hg^0 respectively. The presence of merB and merA in the human gut and their role in MeHg metabolism has not been rigorously investigated. We are therefore exploring the MeHg metabolizing activity of plasmid expressed merB and merA together and in isolation, in *E. coli* cultures. Expression of merB/A together can demethylate and reduce MeHg. Notably, we see that merB alone can accomplish efficient MeHg demethylation, but we observe substantial differences in demethylation rates depending on the species from which the merB is derived. We will investigate the metabolic role of gut-localized MeHg demethylation activity of merB by populating the gut of axenic flies with merB expressing *E. coli*. Results from our study will help to derive controlled assays to elucidate the gut microbiome's role in modulating the toxicokinetics of MeHg and elucidate mechanisms that potentially influence MeHg half-life variability in humans. *Supported by NIEHS R01ES030940 and T32ES007026.*

2574 The Inhibitory Effect of Hexavalent Chromium in Myogenic Differentiation

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Chromium is a metallic element found widely in rocks, soil, plants and animals. Chromium III is a nutritional supplement whose mode of action as such is not well defined. In contrast hexavalent chromium (Cr(VI)) is about 500 -1000 times more toxic than Cr(III) toxic and is an established human carcinogen. Acute exposure to Cr(VI) may affect the skin, kidneys, lungs and gastrointestinal track. Chronic human exposure in occupational settis is associated with nasal and lung cancers. Cr(VI) can cross the placenta and induce development toxicity. Myogenesis is a process of formatting muscular tissue, especially during embryonic development. Since studies in chromium VI induced myotoxic are currently lacking, the aim of the present study was to investigate the toxicological effect of Cr(VI) on C2C12 myoblasts, particularly during myogenic differentiation. We exposed C2C12 myoblast to different doses of Cr(VI) and measured the viability of C2C12 cells. Non-toxicity doses of 2 μm and 5 μm were selected to study the effect of Cr(VI) on myogenic differentiation. We found that Cr(VI) exposures inhibit C2C12 myoblast differentiation in a dose-dependent manner. Consistent with the defective myogenic differentiation, several myogenic differentiation makers, such as MCK, myogenin, myomaker and myomerger, were significantly altered following Cr(VI) treatment. Taken together, our study is the first to evaluate the toxicological effect of Cr(VI) on myoblast differentiation and represents a model system to study the effects of Cr(VI) on human cell differentiation.

2575 Reduction of Erythropoietin Production through Inhibition of HIF-1 Transcriptional Activity by Cadmium

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Cadmium (Cd) is one of the toxic heavy metals widely present in the environment. Cd is contained in grains such as rice and seafood such as oysters. Therefore, there are concerns about the health effects of long-term intake of Cd through these foods. Chronic Cd exposure causes renal toxicity, especially proximal tubular damage. In addition, Cd causes renal anemia. This is mainly due to reduced production of erythropoietin (EPO), one of the hematopoietic factors produced in the kidney. *EPO* is a gene regulated by the hypoxia inducible factor 1 (HIF-1). HIF-1 is known to be a heterodimer consisted of HIF-1 alpha and HIF-1 beta (aryl hydrocarbon receptor nuclear translocator: ARNT). In this study, we examined the effect of Cd on the transcriptional activity of HIF-1 and the gene expression level of EPO. Human kidney proximal tubular epithelial cells (HK-2 cells) were treated with Cd under serum-free conditions.

The transcriptional activity of HIF-1 in HK-2 cells treated with Cd decreased compared to the control. *EPO* mRNA levels were significantly decreased depending on the treatment concentration and treatment time of Cd. Moreover, *EPO* mRNA levels were decreased to approximately 50% in siRNA-treated *HIF-1 alpha* knockdown cells. However, Cd remarkably induced HIF-1 alpha protein levels. On the other hand, ARNT protein levels were decreased in HK-2 cells treated with Cd. These results suggest that Cd may cause renal anemia by reducing EPO production in human kidney proximal tubular epithelial cells through inhibition of HIF-1 transcriptional activity. In addition, Cd may inhibit HIF-1 transcriptional activity via suppression of ARNT protein levels. *This work was supported by the Study of the Health Effects of Heavy Metals, organized by the Ministry of the Environment, Japan.*

2576 Associations between Metal Levels in Whole Blood and IgE Concentrations in Pregnant Women Based on Data from the Japan Environment and Children's Study

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Allergic diseases can potentially affect the course of pregnancy, and poorly controlled allergic diseases during pregnancy are associated with high mortality, low birth weights, and congenital malformations. Various changes occur in pregnant women compared to those who are not pregnant, and pregnant women tend to have higher metal concentrations in their bodies. Recently, the immunotoxic properties of metals have been pointed out and some studies have reported that there are relationships between metal exposures and IgEs. Therefore, we have tried to clarify the relationships between metal concentrations in blood and IgEs in pregnant women. We investigated the relationship between metal concentrations in whole blood and IgEs (total and specific) in 14,408 pregnant women who participated in the Japan Environment and Children's Study. Concentrations of the metals Cd, Pb, Hg, Se, and Mn, as well as serum total and allergen-specific IgEs for egg white, house dust-mites (HDM), Japanese cedar pollen (JCP), animal dander, and moth, were measured. Allergen-specific IgE(s) were divided based on concentrations <0.35 or ≥0.35 UA/mL, and the metal levels were divided into quartiles (Q1-Q4) according to increasing heavy metal levels. There were significant relationships between HDM-specific IgE and Hg Q4; JCP-specific IgE and Hg Q3 and Q4; JCP-specific IgE and Se Q2, Q3, and Q4; animal dander-specific IgE and Hg Q3 and Q4; animal dander-specific IgE and Se Q3; and animal dander-specific IgE and Mn Q2 and Q3. The odds ratio (OR) of HDM- and animal dander-specific IgE decreased as the Hg concentration increased (p values for the trends were 0.010 and 0.006, respectively). Conversely, the OR of JCP-specific IgE increased as the Hg and Se concentrations increased (p values for the trends were both <0.001). This is the first study to link metal concentrations with IgEs in pregnant Japanese women. We showed that metal exposures may be related to both increases and decreases in allergen-specific IgEs and that JCP-specific IgE, in particular, was positively related to Hg concentrations.

2577 Hepatic Processing of Mercuric Ions Is Required for Renal Excretion

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Mercury (Hg) is a prevalent environmental toxicant to which humans are exposed regularly. Renal excretion of mercuric species is the primary means of removal and studies suggest that the primary species of Hg delivered to the kidney is a glutathione conjugate (GSH-Hg-GSH). The route by which Hg becomes bound to GSH is not understood. We hypothesize that mercuric ions within the body must be delivered to the liver where they are conjugated to GSH. Formation of GSH-Hg conjugates may facilitate subsequent renal filtration and excretion. To test this hypothesis, we measured disposition of Hg in control Wistar rats and in rats in which the hepatic artery and portal vein were ligated to prevent blood delivery to the liver. Rats were injected with 0.5 µmol/kg/2 mL $HgCl_2$ containing radioactive Hg and were euthanized after one hour. The amount of Hg in kidneys, liver, and blood was measured. In addition, biochemical assays were used to measure parameters of GSH synthesis. Following exposure to $HgCl_2$, the renal burden of Hg in rats with ligated livers was reduced significantly compared with that of normal rats. Exposure to a GSH-Hg-GSH conjugate increased the renal burden of Hg in rats with ligated livers. Furthermore, exposure to Hg increased the hepatic expression of glutathione-S-transferase. Collectively, these data suggest that mercuric ions are processed by the liver to generate mercuric species (e.g., GSH-Hg-GSH) that can be filtered and excreted by the kidney.

2578 Acute Effects of Inhaled Tungsten Particles on the Lung Microenvironment

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Individuals are exposed to high levels of tungsten through inhalation, particularly in occupational (range: 0.024-3.14 mg/m^3) and military (range: 1.95-3.08 mg/m^3) settings. Inhalation exposure to tungsten metal-alloy particles has been associated with increased incidence of lung pathologies, including interstitial lung disease and lung cancer. However, very little is known about the molecular mechanisms underlying the contribution of tungsten exposure to these pathologies. The present study examined if inhalation exposure to tungsten metal particles caused acute damage to the lung microenvironment that could contribute to these long-term lung pathologies. A total of 24 female Balb/c mice were exposed to control (filtered air), 0.50 mg/m^3 (low concentration), or 1.5 mg/m^3 (high concentration) tungsten metal particles (< 2 µm) in a whole-body inhalation chamber for 4 hrs. Tissue samples were collected at day 1 and day 7 post-exposure for analysis. A significant accumulation of tungsten was measured in the lung and bone post exposure by inductively coupled mass-spectrometry (ICP-MS), but no accumulation was detected in the bronchoalveolar lavage fluid (BALF) or plasma. Additionally, there was a significant increase of macrophage and neutrophil infiltration into the lung following exposure, however no significant changes in total protein in the lung (marker of cellular damage) or changes in peripheral blood counts were observed. The generation of reactive oxygen species (ROS) was evaluated in dissociated lung tissue by DCFDA staining, and no changes in ROS levels were observed following exposure. Interestingly, an increase in the percentage of activated fibroblasts (α-SMA$^+$) in the lung was observed in the high exposure group. Taken together, these findings demonstrate that acute inhalation exposure to tungsten particles, at levels commonly observed in occupational and military settings, resulted in changes to the lung microenvironment that may promote the onset or exacerbation of lung diseases. Current work is focused on exploring these observations through the use of *in vitro* multi-cell lung models and chronic exposure animal models. *Funding: The University of New Mexico College of Pharmacy and INBRE P20GM103451.*

2579 Biomarkers of Acute Kidney Injury in Metal Exposed, War-Injured Veterans: Preliminary Results

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The ongoing conflicts in Iraq and Afghanistan have exposed numerous US service members to trauma from blasts and explosions, oftentimes resulting in retained metal fragments of unknown composition. While high-dose acute exposures to metals such as arsenic and cadmium are widely known to be nephrotoxic, studies have also shown renal injury from chronic, low-level metal exposures. As such, veterans who have on-going systemic metal exposure from embedded fragments may be at risk for kidney injury. To investigate this issue, 421 veterans, identified from the Department of Veterans Affairs' (VA) Toxic Embedded Fragment registry, across 5 VA medical centers are being asked to complete medical history and exposure questionnaires along with spot urine collection. We present here preliminary data for the first 119 enrolled veterans. Urine samples were analyzed for levels of 14 metals commonly found in fragments or known to be carcinogenic, along with 10 protein markers of kidney injury. All urine metal results were creatinine-adjusted and dichotomized based on the upper limit of normal in unexposed populations. Linear regression was used to examine the effect of having at least one elevated metal concentration on renal protein markers while controlling for other factors associated with renal injury. The study population was predominantly male (96.6%) with a mean age of 41.2 years and average time since injury of 12.4 years. Over half (56.3%) of veterans had at least one elevated urine metal, the most common of which were zinc (49.6%), aluminum (6.7%), and tungsten (3.4%). Except for interleukin-18, the majority of renal protein results fell within normal ranges. Initial findings showed that the presence of an elevated urine metal concentration was significantly associated with seven of ten renal injury markers after adjustment for age and NSAID use. However, concerns about the unusual number of elevated zinc results prompted removal of zinc from the analyses. After zinc removal, only 23 (19.3%) veterans had at least one elevated urine metal concentration and

a significant association with 3 renal injury markers remained (N-acetyl-β-D-glucosaminidase, total protein, and albumin). While most renal protein results fell within the normal range, our preliminary findings suggest that long-term monitoring to assess adverse renal effects may be warranted in veterans with retained fragments.

2580 Exposure to Lead and Cadmium, and Health Effects in Abandoned Metal Mine Area Inhabitants of Korea

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People living near abandoned mine area are at risk of metal exposure. In this study, we surveyed 4500 inhabitants (male: 1768, female: 2732) around 104 abandoned metal mines during 2013-2017 (the 2nd phase health survey in Korea). The mean age of study subjects were 68.5 years-old. The concentrations of lead (Pb) and cadmium (Cd) in whole blood and Cd in urine were analyzed by a graphite furnace atomic absorption spectrometer. The geometric mean of BPb, BCd and UCd were 2.27 μg/dL (male: 2.71 μg/dL, female: 2.03 μg/dL), 1.42 μg/L (male: 1.18 μg/L, female: 1.60 μg/L), and 1.66 μg/g creatinine (male: 1.13 μg/g creatinine, female: 2.13 μg/g creatinine), respectively. Exposure level to metals was lower than the 1st phase health survey with 38 abandoned mines in Korea (2008-2011), but was higher than the general population. BPb was significantly higher in males than in females while BCd and UCd were significantly higher in females than in males. In this study, BPb was the highest in the age group of 40-59 then decreased, while BCd and UCd were increased by the eighties. Furthermore, negative relations observed between BPb and Cd in blood and urine in this study subjects. These findings indicate that abandoned mine area inhabitants have been exposed to Cd continuously even though the mines were closed several decades ago. Also, 252 of 4500 subjects exceeded the threshold of BCd or UCd, and some of them also observed health effects such as kidney and bone damage. Taken together, inhabitants in abandoned mine area are aged mostly and might be susceptible to toxic metal such as Cd, therefore, it is necessary continuous monitoring and careful attention in environmental health.

2581 Inhibition of Red Blood Cell Development by Arsenic-Induced Disruption of GATA-1

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Anemia is a hematological disorder that adversely affects the health of millions of people worldwide. Although many variables influence the development and exacerbation of anemia, one major contributing factor is the impairment of erythropoiesis. Normal erythropoiesis is highly regulated by the zinc finger (ZF) transcription factor GATA-1. Disruption of the ZF motifs in GATA-1, such as produced by germline mutations, compromises the function of this critical transcription factor and causes dyserythropoietic anemia. Herein we report that arsenic, a widespread environmental toxicant, selectively inhibits erythropoiesis through the disruption of GATA-1 ZFs, utilizing a combination of *in vitro* and *in vivo* studies. We found that arsenic interacts with the N- and C-terminal ZF motifs of GATA-1, causing zinc loss and inhibition of DNA and protein binding activities, resulting in dyserythropoiesis and an imbalance of hematopoietic differentiation. For the first time, we show that exposures to a prevalent environmental contaminant compromises the function of a key regulator of erythropoiesis, producing effects functionally similar to inherited GATA-1 mutations. These findings highlight a novel molecular mechanism by which arsenic exposure may cause anemia and provide critical insights into potential prevention and intervention for arsenic-related anemias. *This work was supported by the National Institutes of Environmental Health Sciences R01 ES029369 (K.J.L), R01 ES019968 (S.W.B.), and University of New Mexico Clinical and Translational Science Center Grant Number UL1TR001449 (S.W.B.).*

2582 Comparison of Toxicokinetics of Methylmercury in Diabetic KK-Ay Mice and C57BL/6 Mice

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One modifier of methylmercury (MeHg) metabolism and its toxicokinetics is the metabolic disturbance associated with a disease. However, the toxicokinetics of Hg when disturbed by glucose metabolism have not yet been clarified. The present study aimed to elucidate the differences in MeHg toxicokinetics when disturbed by glucose metabolism between diabetic KK-Ay mice and non-diabetic BL/6 mice using the Kp value, the equilibrium ratio between the drug concentrations in the organ and the plasma. A single dose of MeHg (0.2 mg Hg/kg BW) was administered to 12-week-old KK-Ay and BL/6 mice. After 4, 7, 11, and 14 d, the total mercury (T-Hg) concentration in samples of blood and tissue (brain, kidney, liver, and pancreas) was determined using a thermal decomposition Hg analyzer. The Kp value in the brain samples of the KK-Ay mice tended to reach a plateau after 11 d, but that in samples from the BL/6 mice continued to increase up to 14 d. The Kp value in the liver in both the KK-Ay and BL/6 mice tended to reach a plateau at 11 d. The Kp value in the kidneys of the KK-Ay mice remained almost constant from 4 to 14 d, but that in samples from the BL/6 mice continued to increase up to 14 d. The Kp value in the pancreas of the KK-Ay mice decreased continuously up to 14 d, but that in samples from the BL/6 mice remained almost constant from 4 to 14 d. These findings indicated that the uptake and excretion of MeHg in each organ differed between the diabetic KK-Ay mice and the non-diabetic BL/6 mice. These results will be useful for understanding MeHg metabolism when glucose metabolism is disturbed by a disease such as diabetes.

2583 Detoxification Mechanisms in Nematode *Caenorhabditis elegans* Exposed to Nickel

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Occupational and environmental exposure to nickel has been known to be carcinogenic and allergenic in human populations. Developing methods for protecting organisms in nickel-polluted environments settings is contingent upon the understanding of cellular detoxification mechanisms. *Caenorhabditis (C.) elegans* is a powerful model organism for investigating toxicological processes, and provides several advantages for deciphering the mechanisms of metal detoxification. *C. elegans* harnesses multiple detoxification mechanisms to maintain metal homeostasis. Members of the heavy metal tolerance factor 1 (HMT-1) which belong to the "B" branch of the ATP-bindings cassette (ABC) transporters superfamily, are required for detoxification of heavy metals. Phytochelatins (PC) are a class of glutathione-derived peptides that maintain metal homeostasis via binding metal ions. The enzyme, phytochelatin synthase 1 (PCS-1), synthesizes PC in response to toxic metal exposures. The pcs-1 and hmt-1 genes are co-expressed in the nematode's coelomocytes, which act as liver cells by reprocessing and detoxifying pseudocoelomic fluid from harmful ingested substance. The objective of this study was to investigate roles of the metal-detoxifying mechanisms described above in nickel tolerance in *C. elegans*. The wild-type N2 nematodes, single knockout strains, VF2 (pcs-1 null) and VF3 (hmt-1null), and double knockouts strains, VF9 (pcs-1+hmt-1) and VF14 (hmt-1+coelomocytes), were exposed to a series of concentrations of nickel sulfate (0 to 500 μmol/L). The high-throughput platform analysis (COPAS Biosort and WMicroTracker) were used to measure different endpoints, including growth, reproduction, feeding, and locomotion. Following exposure nickel sulfate induced significant toxic effects on growth, reproduction, feeding, and locomotion in exposed wild-type *C. elegans*. Significant differences were found between the wild type and mutant strains in response to nickel exposure. The EC50 of locomotion in wild type N2, mutant VF2, VF3, VF9 and VF14 were 36.46, 30.44, 23.39, 21.94 and 18.96 μM, respectively. These data suggest that pcs-1 and hmt-1 pathways and coelomocytes are involved in nickel detoxification, which may be used as a novel model for studying nickel-induced toxic effects and mechanisms.

2584 Assessing the Effects of Cr(VI) on CTCF Binding and Localized Transcription Dysregulation

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Hexavalent chromium (Cr(VI)), is a well-established respiratory carcinogen widely used in several industries. While inhalation is a main source of exposure in occupational settings, millions of people are chronically exposed through ingestion of contaminated drinking water, creating an increased risk of gastrointestinal cancer, though the mechanisms remain unclear. Our previous findings using ATAC- and MNase-seq demonstrated that Cr(VI) exposure resulted in differentially accessible chromatin regions enriched with motifs for CTCF, a key regulator of transcription and chromatin architecture. In our current study, we used ChIP-seq to test the hypothesis that Cr(VI) disrupts the interaction of CTCF with its cognate motifs, altering proximal chromatin architecture and regulation of gene expression. Following exposure to 1 μM Cr(VI) for 48 hours, we identified differentially bound sites containing the motif, with the trend favoring reduced CTCF binding in the presence of Cr(VI). The mRNA and protein expression of CTCF and cohesin components were not appreciably affected, suggesting that disrupted binding affinity is the result of Cr-mediated interference of CTCF interaction with chromatin. To assess the impact on local transcriptional regulation, we selected for motifs within ±1.5 Kb of a transcriptional start site, mapping the closest gene's expression using a previously published RNA-seq dataset. Functional enrichment analyses for significantly affected genes identified key factors in chromatin organization and chromatin modifying enzymes, most notably arginine methyltransferases, supporting the notion that Cr promotes changes in chromatin architecture. To better understand how architectural shifts affect gene expression profiles, we are attempting to identify Cr-susceptible chromatin loops within topologically associated domains (intraTAD) by applying a machine learning approach to a multifaceted ChIP-seq dataset of transcription factors and histone modifications. Our findings suggest that Cr(VI) exposure disrupts the interactions of CTCF with a subset of its binding sites, either directly or indirectly, which may alter the transcription of genes within functional networks associated with chromatin organization. *Acknowledgements: Supported by NIEHS R01ES010807 and NIH 5T32ES007250.*

2585 Diffusion Mechanism, Equilibrium, and Kinetics of Nickel (II) Ions Adsorption onto White Croaker Fish Scales

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The absorption removal of Ni (II) ions from aqueous solution was investigated by purverised white croaker fish scale (FS) in a batch process. The FS was characterised by Fourier transform infrared (FTIR) before and after the adsorption. The optimum conditions and % adsorption of the Ni (II) ions were found to be the following: pH 7.0; 94.1%, adsorbent dose 20 g/L; 80%, concentration 50 mg/L; 88.3%; contact time 90 mins; 89% and temperature 323 K; 93% respectively. The equilibrium adsorption data was applied to Langmuir, Freundlich, Temkin, and Dubinin-Kaganer-Raduskevich (DKR). The equilibrium was fully fitted with Langmuir and the dimensionless separation factor R_L signifies a favourable adsorption of Ni (II) ions FS. The adsorption kinetics studies reveal that the adsorption of Ni (II) ions onto FS was pseudo-second order chemisorption and follow film diffusion as well as intra-particle pore diffusion mechanism. Thermodynamics study showed that the adsorption is endothermic and spontaneous. The study shows the potential capacity of fish scale in the effective removal of Ni (II) ions from aqueous solution.

2586 Metabolism Shift in Cr(VI)-Transformed Cells Causes Epigenetic Changes and Promotes Tumorigenesis

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Cancer cells display a metabolic shift from oxidative phosphorylation to glycolysis under normoxia condition. Metabolic shift is important for the production of anabolic building blocks for developing hallmarks of cancer. Certain metabolites produced from shifted metabolism could function as substrates or regulators for epigenetic modifiers. Hexavalent chromium (Cr(VI)) chronic exposure induces malignant transformation of human bronchial epithelial (BEAS-2B) cells, displaying cancer stem cell (CSC)-like property and tumorigenesis. This study investigated whether Cr(VI)-transformed cells display abnormal metabolism and its role in Cr(VI)-induced CSC-like property and tumorigenesis. It was found that compared to passage-matched control cells, Cr(VI)-transformed cell [BEAS-2B-Cr(VI)] displayed glycolytic shift, Which could be reversed under a no glucose culture condition. Moreover, culturing BEAS-2B-Cr(VI) cells in no glucose medium impaired their CSC-like property and tumorigenesis. Mechanistic studies revealed that the levels of histone acetylation marks are up-regulated in BEAS-2B-Cr(VI) cells. The expression level of ACLY (ATP Citrate Lyase), a gene that produce acetyl co-A for histone acetylation, is drastically increased in BEAS-2B-Cr(VI) cells. Moreover, the expression level of c-Myc, a gene that plays critical roles in cancer stemness and metabolism, was also increased greatly in Cr(VI)-transformed cells. Cr(VI)-transformed cell cultured under no glucose condition showed reduced levels of ACLY, c-Myc and histone acetylation marks. Moreover, inhibition of ACLY with a pharmacological inhibitor greatly reduced the levels of histone acetylation marks and c-Myc in Cr(VI)-transformed cells and their CSC-like property. Knockdown of c-Myc in Cr(VI)-transformed cells reversed their glycolytic shift, histone acetylation, CSC-like property and tumorigenesis. Together these findings suggest that metabolic shift in Cr(VI)-transformed cells increases histone acetylation through ACLY, which in turn up-regulates the expression of ACLY and c-Myc establishing a positive feedback loop between metabolic shift and epigenetic deregulation and promoting CSC-like property and tumorigenesis.

2587 Cadmium Renal Toxicity through the Suppression of Gene Expression of GLUT4

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Cadmium (Cd) causes renal toxicity through the proximal tubular cell damage. Previous studies revealed that Cd caused the gene expression disruption in proximal tubular cells. However, the transcription factors that regulate the expression of genes associated with Cd renal toxicity are poorly understood. We found MEF2A (myocyte enhancer factor 2A) transcription factor which is involved in Cd toxicity in HK-2 human proximal tubular cells, using protein/DNA binding array and RNAi method. Next, it was investigated which downstream factor of MEF2A is associated in Cd toxicity in HK-2 cells. MEF2A is reported to regulate the gene expression of *SLC2A4*, which encodes GLUT4 (glucose transporter 4) protein. We found that *SLC2A4* expression is regulated by MEF2A in HK-2 cells. Moreover, Cd decreased not only the mRNA level of *SLC2A4* but also the protein level of GLUT4 in HK-2 cells. In addition, *SLC2A4* knockdown by siRNA conferred the toxicity to HK-2 cells. On the other hand, GLUT2 protein is reported to be expressed abundantly in the mammalian kidney. However, the knockdown of the GLUT2 encoding gene, *SLC2A2*, did not induce the toxicity in HK-2 cells. Furthermore, *MEF2A* knockdown did not inhibit the gene expression of *SLC2A2* in HK-2 cells. GLUT4 is involved in glucose transport into the cells. Therefore, it was examined whether Cd or *SLC2A4* siRNA treatment affect the glucose level in HK-2 cells. Not only Cd treatment but also *SLC2A4* siRNA treatment significantly decreased glucose level in the cells. Furthermore, HK-2 cells incubated in glucose free medium showed the decrease in cell viability compared to that incubated in normal medium. These results suggest that the pathway of Cd renal toxicity through the inhibition of the MEF2A activity involves the cellular glucose level decrease *via* the suppression of *SLC2A4* gene expression.

2588 Integrin α4 Depletion Suppresses Arsenic and Benzo[α]pyrene Co-exposure-induced Cancer Stem Cell-Like Property by Inhibiting the Non-canonical Hedgehog Pathway

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Arsenic (As) and benzo[α]pyrene (BaP) are among the most common environmental carcinogens causing lung cancer in humans; and As and BaP co-exposure synergizes in inducing lung tumorigenesis. However, the mechanism of the synergistic carcinogenic effect of As and Bap co-exposure remains elusive. The goal of this study was to investigate the mechanism of As and BaP co-carcinogenesis. We found that As and Bap co-exposure synergizes in inducing cancer stem cell (CSC)-like property, which is stronger than that induced by exposure to As or BaP alone. It was further determined that the expression level of integrin α4 (ITGA4) is highly up-regulated in As and Bap co-exposure-transformed human bronchial epithelial cells [BEAS-2B-A+B]. To determine the role of ITGA4 up-regulation in As and BaP co-exposure-induced CSC-like property and tumorigenesis, we stably knocked down ITGA4 expression in BEAS-2B-A+B cells using ITGA4 targeting shRNA lentiviral particles. It was found that stably knocking down the expression of ITGA4 in BEAS-2B-A+B cells significantly decreased their CSC-like property and tumorigenicity in mice. Mechanistic studies revealed that Hedgehog (HH) pathway, a master

regulator of developmental processes whose dysregulation has been linked to a variety of cancers, was turned off in ITGA4 knock down BEAS-2B-A+B cells. It was further determined that ITGA4 knock down suppresses non-canonical HH signaling pathway by decreasing the expression and nuclear localization of activator Gli (GLI^A). Moreover, inhibition of the HH pathway significantly reduced the CSC-like property of As and BaP co-exposure-transformed cells. Together, these findings indicate that ITGA4 depletion suppresses As and Bap co-exposure-induced cancer stem cell-like property by inhibiting the non-canonical HH signaling pathway.

2589 Comprehensive Study on the Target Transcription Factors in Cadmium Renal Toxicity

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Cadmium (Cd) is an environmental contaminant known to exert toxic effects on various tissues such as kidney, liver, lung, testis and bone. Especially, proximal tubular cell damage is characterized as Cd-induced renal damage. Previous studies revealed that the disruption of gene expression is involved in Cd-induced renal toxicity. However, the transcription factors that regulate the expression of genes associated with Cd renal toxicity are poorly understood. In this study, we screened transcription factors whose activities were changed by Cd treatment in not only HK-2 human proximal tubular cells but also NRK-52E rat proximal tubular cells, using Protein/DNA binding array. In HK-2 cells, Cd increased the binding activities of 20 transcription factors by more than 2-fold; also, Cd decreased those of 28 transcription factors, among 345 transcription factors. In addition, in NRK-52E cells, Cd increased the activities of 6 transcription factors by more than 2.0-fold and decreased those of 15 transcription factors by less than 0.5-fold, among 65 transcription factors. We focused on the transcription factors whose activities were decreased by Cd; therefore, it is examined which transcription factors might decrease cellular viability by their knockdown using the RNAi method. As the result, the each knockdown of ARNT, MEF2A, HIF-1, GATA-1, GATA-3, GATA-6, FOXF1, and YY1 transcription factors conferred the cell viability defection. Interestingly, several downstream factors such as BIRC3 [baculoviral IAP (inhibitor of apoptosis protein) repeat containing 3; known as cIAP2] and UBE2D (ubiquitin-conjugating enzyme E2D) are involved in apoptosis related Cd toxicity. These results suggest that the gene expression disruption through the changes in transcription activities is one of the main pathways in Cd renal toxicity.

2590 Humanizing the *Borcs7/As3mt* Locus Establishes Human-Like Patterns of Arsenic Metabolism in Mice

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Both mice and humans metabolize inorganic arsenic (iAs) via reactions catalyzed by arsenic (+3 oxidation state) methyltransferase (AS3MT/As3mt) to yield monomethyl-As (MAs) and dimethyl-As (DMAs) metabolites. However, mice metabolize iAs more efficiently than humans, resulting in a faster clearance, which may explain why some of the adverse effects of iAs exposure have been difficult to reproduce in laboratory mice. The goal of the present study was to generate a mouse strain in which iAs metabolism resembles that in humans. Embryonic stem cells (ESCs) expressing human *AS3MT* were created by syntenic replacement of the mouse *Borcs7/As3mt* locus with human the *BORCS7/AS3MT* locus. ESCs carrying the humanized locus were used to generate the *AS3MT/BORS7* (hu/hu) humanized mouse line. The concentrations of iAs and its metabolites were measured in urine of wild-type (WT) and hu/hu mice after a single oral dose of iAs (20 ng/g b.w.) and urine and tissues after a 4-week exposure to 400 ppb iAs in drinking water. Hu/hu mice excreted 20% (males) and 35% (females) of the oral dose over 72 hours as compared to 51% and 76% excreted by WT mice. DMAs represented >97% of total As (tAs) in urine of WT mice, but only 40-60% in urine of hu/hu mice (iAs and MAs accounted for 29-54% and 10-17%, respectively). Similar differences in the proportions of As species were found in urine of WT and hu/hu mice exposed to iAs for 4 weeks; tAs concentration was lower in urine of hu/hu mice. After 4 weeks, livers of hu/hu mice contained 20 times (males) and 30 times (females) more tAs than livers of WT mice. iAs and MAs represented 80-86% and 14-20% of tAs in livers of hu/hu mice, respectively, while DMAs accounted for 85-95% of tAs in livers of WT mice. Significant differences in tAs levels and proportions of As species were also found in kidneys; iAs represented 40-50% and MAs 45-55% of tAs in kidneys of hu/hu mice, but only 10-20% and 4-9% in kidneys of WT mice. In summary, iAs metabolism (detoxification) in hu/hu mice is less efficient than in WT mice, resembling the rate and patterns of iAs metabolism in humans. Thus, this humanized mouse strain may be a more adequate model for laboratory studies aiming to reproduce in mice the adverse effects of iAs reported in humans. *This abstract does not reflect US EPA policy.*

2591 Methylmercury-Induced Lipid Accumulation in *Caenorhabditis elegans* Is Dependent on microRNA Expression

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Dyslipidemia is a hallmark of metabolic conditions, such as obesity. Epidemiologic data support an association between blood mercury levels with obesity, lipid dysregulation, and increased visceral adipose tissue. In obesity, activation of adipogenic transcription factors and expression of adipogenic microRNAs (miRNAs) drive the hypertrophy and hyperplasia of adipocytes. We have previously shown that methylmercury (MeHg) increases the expression of adipogenic transcription factors in *Caenorhabditis elegans*; however, there is limited information on how MeHg affects microRNA profiles. We investigated whether altering miRNA expression could prevent lipid accumulation in the worm. Worms that express mutations in *rrf-1* or *rrf-3* are more sensitive to the effects of miRNA, while worms that express mutant *pash-1* or *nrde-2* are more resistant to miRNA than wild type (N2) worms. miRNA sensitive strains were more resistant to MeHg toxicity than N2, while the miRNA resistant strains were more sensitive to MeHg toxicity. These data suggest that the worms express pro-survival miRNA in response to MeHg, which is amplified in the sensitive strains, but absent in the resistant strains. miRNA sensitive strains accumulated less triglycerides and fat deposits than N2 worms. In contrast, miRNA resistant strains had exacerbated triglyceride levels and fat deposition than N2. These data suggest that anti-adipogenic miRNA are enriched in the miRNA sensitive strains, while they are prevented from altering metabolism in the miRNA resistant strains. Behaviors associated with fat accumulation were assessed in the transgenic worms exposed to MeHg. MeHg increases food consumption in N2 worms, which was prevented in the miRNA sensitive worms. Furthermore, miRNA sensitive worms showed improved locomotion and foraging behaviors than the N2 or miRNA resistant worms exposed to MeHg. Together, our data suggest that miRNA expression play important roles in survival responses and lipid homeostasis following MeHg exposure.

2592 Renal Anemia in *itai-itai* Disease: A Case Series Study of Medical Records of the Patients

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Sponsor: M. Satoh

Itai-itai disease (IID), characterized by renal tubular dysfunction and osteomalacia, is the most severe form of chronic cadmium (Cd) poisoning developed among female farmers in the heavily Cd-polluted Jinzu River Basin in Japan. Recent researches demonstrated that the patients often suffer from renal anemia, derived from insufficient production of erythropoietin (EPO) from the kidney. In order to clarify the clinical feature of renal anemia in IID, we performed a case series study on the patients with IID, especially in reference to anemia, using electronized archives prepared from their medical records preserved in Hagino hospital. From 288 cases in the archives, we picked out 283 ones (23 males and 260 females, mean age 74.6±8.4 years) whose peripheral blood hemoglobin (Hb) data were available. We collected their clinical data, including age, sex, Hb, erythrocyte counts, hematocrit, serum iron and ferritin levels, history of treatment, etc. Because Hb levels were measured by a cyanmethemoglobin method (in 1975 and after then) or a Sahli method (in 1975 and before then), we analyzed the cases separately by the methods to measure Hb. More than 95% of the cases were suffered from anemia, and about 60% of female cases in 1975 and after then were judged as severe anemia (less than 7 g/dL of Hb). There was no significant relationship between Hb and age. There were 16 cases whose serum EPO levels were measured. Among them, 14 cases were judged as renal anemia, demonstrated by the low levels of serum EPO despite low levels of Hb. However, because there were quite a few cases showing macrocytic or hypochromic anemia, additional mechanisms, such as insufficiency of vitamin B_{12} or iron, might be involved in the development of anemia in IID. According to history of treatment in the cases with renal anemia, EPO substitution therapy was effective for recovery of erythropoiesis, but iron deficiency attenuated its effect. These results indicate that renal anemia, often advancing in severity, is a very important intrinsic clinical sign in IID, and EPO substitution therapy along with iron supplement is recommended for renal anemia in patients with IID.

2593 Sodium Arsenite Alters Glucose Regulation-Associated Transcripts in Sperm Cells of C57BL/6J Mice

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Exposure to inorganic arsenic (iAs) is associated with both cancer and other diseases such as diabetes in both humans and rodents. In mammals, prenatal exposure to iAs is associated with alterations in the epigenome that are associated with adverse birth outcomes or increased disease risk later in life. In terms of an underlying mechanism, studies have identified altered gene expression and DNA methylation changes as potential mechanisms tied to iAs-driven health effects. Although the effects of prenatal iAs exposure has been assessed, the contribution of preconception exposure to disease risk in offspring has never been systematically studied. In this study, we hypothesized that sub-chronic preconception exposure to iAs would lead to changes in transcript levels regulated through epigenetic mechanisms in parental germline cells that will be inherited by offspring. Adult male and female C57BL/6J mice were exposed to sodium arsenite (0 or 200 ppb) in drinking water for 10 weeks. On week 10, fasted blood glucose and fasted plasma insulin levels were measured and showed no treatment-related effects. iAs treatment was terminated prior to mating and the female mice were closely monitored for pregnancies. Animals from the parental generation were euthanized either prior- or post-mating period. RNA-sequencing was conducted in oocytes and sperm cells from the parental generation. Transcript levels of protein tyrosine phosphatase non-receptor type 2 (*Ptpn2)* were significantly upregulated and gene expressions of endosialin (*Cd248)*, chemokine ligand 8 (*Ccl8)* were found to be significantly down-regulated in sperm cells with iAs exposures. In addition, expression of major vault protein (*Mvp*) and Ras-Related Protein Rab-3D (*Rab3d)* mRNAs were also found to be differentially expressed in relation to exposure. Pathway enrichment analysis in sperm cells revealed perturbation of extracellular matrix (ECM) signaling, protein kinase B, insulin-like growth factor binding, and other pathways that are associated with glucose dysregulation. Results of small-RNA and DNA methylation analyses in sperm cells are currently being analyzed. In summary, these data help address molecular mechanisms underlying iAs-associated disease in response to preconception exposure. A follow-up study will be conducted in the offspring from this study to investigate the relationship to health outcomes.

2594 A Mechanism Underlying the Enhancement of Methylmercury Toxicity through Activation of Transcription Factor HOXB13

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Excessive consumption of methylmercury-contaminated fish cause severe central nervous system injury, such as in Minamata disease. Recent epidemiological studies have suggested excessive intake of methylmercury during pregnancy to have an adverse effect on the fetal neural development. However, the molecular mechanisms underlying methylmercury-induced central nervous system injury are still only poorly understood. We recently found that homeobox protein B13 (HOXB13), a transcription factor, is related to methylmercury toxicity; however, the downstream factor involved in enhancing methylmercury toxicity remains unknown. To elucidate the mechanisms involved in the enhancement of methylmercury toxicity by HOXB13, we searched the genes whose expression is induced by methylmercury via HOXB13, using a microarray analysis, and then examined the relationship between a gene, in which some change was recognized after HOXB13 knockdown, and methylmercury toxicity. Methylmercury induced the expression of oncostatin M (OSM), a cytokine belongs to interleukin-6 family, and this was remarkably suppressed by HOXB13 knockdown. OSM knockdown also conferred resistance to methylmercury in HEK293 cells, and no additive methylmercury resistance was observed when both HOXB13 and OSM were knocked down. The binding of HOXB13 to OSM gene promoter was increased by methylmercury, indicating the involvement of HOXB13 in the enhancement of its toxicity. Since addition of recombinant OSM to the medium enhanced methylmercury toxicity in OSM-knockdown cells, extracellularly released OSM was considered to enhance methylmercury toxicity via membrane receptors. We discovered TNF receptor 3 (TNFR3) as the potential candidate involved in the enhancement of methylmercury toxicity by OSM. Overall, we report, for the first time, that HOXB13 is involved in enhancement of methylmercury toxicity via OSM expression induction, and that the synthesized OSM causes cell death by binding to TNFR3 extracellularly.

2595 Oral and Inhalation Bioaccessibility of Cobalt and Nickel in Metal Alloys: A Critical Consideration for Site-Specific Human Health Risk Assessments and Read Across

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For risk assessment, nickel and cobalt are evaluated as specific chemicals or pure metal because bioavailability and toxicity vary by extracelluar and intracellular ion solubility. However, nickel and cobalt in alloys, such as stainless steel, do not fit these paradigms because ion solubility may be highly limited by an impervious chromium oxide layer that forms on the surface. Hence, metal ions are not readily released from alloys into biological fluids (limited bioaccessibility and bioavailability). *In vitro* bioaccessibility (IVBA) or bioelution is a test method based on simulated biological fluids to estimate *in vivo* conditions. Arsenic and lead in soil have been tested extensively; IVBA has been used by US regulators for risk assessment and is also under consideration by ECHA for read across. However, for cobalt and nickel, limited *in vitro* and *in vivo* data are available. To expand the database of cobalt and nickel in alloy impacting environmental media, we conducted inhalation and oral IVBA studies using simulated lysosomal and gastric fluids to estimate bioaccessibility of cobalt and nickel in media from 3 facilities in the US Thirty-nine samples of soil, dust, and baghouse dust were tested. Cobalt and nickel content in the samples ranged from 16 to 14,000 mg/kg and from 16 to 45,000 mg/kg, respectively. In simulated lysosomal fluid, bioaccessibility of cobalt and nickel were 4.7% to 21.1% and 3.9% to 10.8%, respectively. In simulated gastric fluid, bioaccessibility of cobalt and nickel were 0.7% to 12.2% and 0.2% to 26%, respectively. Bioaccessibility of cobalt and nickel were inversely related to the cobalt and nickel content in the samples; the results were consistent with a previous bioaccessibility study that we conducted. These data add to the weight of evidence that metals in alloys are resistant to bioelution, have relatively lower potential for absorption as compared to the forms used in animal bioassays, which are the basis of toxicity criteria. Our studies further confirm that bioelution methods should be used for read across and in human health risk assessment.

2596 Integrating Physiologically Based Kinetic Models with Transcriptomics to Support Risk Assessment in Toxicology

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Transcriptomics is a powerful tool in safety assessment which is used to gain a better understanding of the adverse effects associated with chemical exposures in humans and animals. This technique allows for the identification of toxic specific biomarker genes and to follow their expression changes over time. In this study, we use physiologically-based kinetic (PBK) models in combination with transcriptomics data to explain differences in expression patterns over time and between species. In brief, human and rat *in vivo* and *in vitro* data from kidney and liver tissues, exposed to metals, cadmium and lead was selected and subjected to analyses. Focus was put on transporters and metallothioneins(Mts) genes, to see how dose dependent cadmium correlation to Mts can be reflected in these genes' transcriptomics profiles and how transporter proteins influence the expression pattern of their corresponding genes affecting the absorption, distribution and excretion of these metals. The results indicate that PBPK based simulations of validated cadmium and lead exposure scenarios can be linked to the time profile of transcriptomics analyses. Differences in chemical accumulation between tissues and species indeed help explain the variation in the gene expression levels. The effectiveness of this integrative approach makes PBK models more dynamic which can further aid chemical safety assessment by effectively identifying genes playing a role in disease ontology.

2597 Hematological Profile and Mercury Exposure in Adolescents from the Colombian Caribbean

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The blood is a tissue susceptible to cytotoxic agents, in particular, heavy metals such as mercury (Hg). High levels of Hg in humans have received special attention due to the extensive number of adverse effects documented on many systems. In this context, adolescents are a vulnerable group, since some organs are still undergoing development. In general, one of the main routes

of human exposure to Hg is through the consumption of contaminated fish. Therefore, fishing communities located in coastal areas with known Hg releases, for instance, from extinct chloralkali factories, are at risk of developing health problems. The purpose of this research was to study the relationship between hematological parameters and Hg exposure in adolescents living on Tierrabomba Island, a place located near an industrial zone, and in an age-matched group from San Onofre, a distant, non-industrial, coastal community, used for comparison purposes. Hematological parameters were evaluated using an automatized equipment and total Hg in hair was measured employing a direct Hg analyzer. There were significant differences between group means for almost all parameters measured in red blood cells, with greater values found in Tierrabomba, except for RDW. For the white line cell, leucocytes and granulocytes displayed higher values in San Onofre, whereas lymphocyte and monocyte counts were bigger in Tierrabomba. Volunteers from the reference area had mean hair Hg concentrations larger than those from Tierrabomba Island, although in both sampling sites more than 49% of participants had Hg concentrations above international guidelines. For all participants, Hg levels were negative correlated with mean corpuscular hemoglobin concentration (r=-0.162, p=0.024). In the industrial site, Hg correlated with the percentage of monocytes (r=0.193, p=0.041), while in the reference site the association was registered with mid-sized cells (r=0.223, p=0.044). These results suggest that even a low Hg exposure could alter the hematological profile in adolescents. *Colciencias-UniCartagena. Grants 778, 2017 (110777757883); 727, 2015.*

PS 2598 Comparison of Atomizer Design and Concentration of Metals/Elements in Aerosols from Three Generations of Electronic Cigarettes

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Since their introduction in 2004, electronic cigarettes (EC) have evolved into three distinct generations of devices, which include the cig-a-likes, clearomizers, and mods. The atomizer, which heat the e-fluid, contains various metal components and is important as it affects the performance of ECs and what transfers into the aerosol. The purpose of this study was to examine the elements/metals in EC atomizers and the elements/metals in EC aerosols as a function of evolution, puffing topography, and collection method. Data were consolidated on atomizers from 32 brands representing three generations of ECs (cig-a-likes including cartomizers and disposables, clearomizers, and mods). The atomizers were dissected and photographed using a stereoscopic microscope, then elements in atomizer components were analyzed using scanning electron microscopy and energy dispersive spectroscopy. The concentration of 19-37 elements/metals in aerosols was determined using inductively coupled plasma optical emission spectroscopy. EC atomizers across all generations contained a filament(s) (usually nichrome) and wicks (silicon, cotton). Other major components were: a thick wire (copper, nickel), joints between wires (tin/lead solder, brass clamps, brazing of wires, a sheath(s) (fiberglass)), and Polyfil fibers. Some third-generation models of EC lacked several of these components in their atomizers. Of 37 elements studied, 12 (aluminum, calcium, chromium, copper, iron, lead, magnesium, nickel, silicon, sodium, tin and zinc) were frequently present in EC aerosols across all three generations. Calcium, silicon, and tin often had the highest concentrations (0.012-1.124 mg/L, 0.021-5.581 mg/L, and 0.001-2.645 mg/L, respectively). For some elements, such as arsenic, copper, iron, nickel, lead, tin, the concentration increased in aerosols as voltage/power increased with the introduction of clearomizers and mods. Many of the elements detected in the aerosols originated from components in the atomizers. Concentrations of some elements, such as chromium, lead, and nickel, were higher in aerosols of the later generation ECs and may present a health risk.

PS 2599 Cadmium Accumulation and Effects in Insulin-Producing Beta Cells in the Context of Type 2 Diabetes

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Introduction: Failure of insulin-producing beta-cells in pancreatic islets of Langerhans in the setting of insulin resistance is the underlying cause for type 2 diabetes mellitus (T2DM). Only a third of individuals with significant insulin resistance develop beta-cell failure and diabetes. Genetic susceptibility, lifestyle, and environmental factors contribute to beta-cell failure in T2DM. Environmental contributors toward beta-cell failure in type 2 diabetes mellitus are underexplored. Others and we previously provided evidence for a correlation between cadmium (Cd) exposure and T2DM as well as the accumulation of cadmium in insulin-producing pancreatic beta-cells. We also previously described the presence of a relatively high concentration of Cd in primary insulin-producing islets of Langerhans isolated from the general US population that is correlated with age, supporting prior evidence of a gradual tissue accumulation over time. Furthermore, we found an avid uptake of Cd in isolated insulin-producing beta-cells, likely related to their high endowment of zinc (Zn) transporters and high Zn turnover. In order to recapitulate the effect of chronic Cd exposure on beta-cells *in vivo*, we established murine oral Cd exposure model for the study of Cd-induced dysglycemia. Herein, we report our current findings related to this effort. Methods: C57Bl/6N mice were exposed to $CdCl_2$ or vehicle in drinking water. Obesity and insulin resistance were achieved by placing the animals on high fat diet (42% calories from fat). Results: We established that an oral $CdCl_2$ exposure protocol that consists of a loading period with 0.5mM $CdCl_2$ in drinking water for 120 days followed by a washout period of 90 days yielded blood and islet Cd concentrations within the range found in human blood and islets, thereby representing a relevant islet Cd concentration. In this model, we found significant impairment of insulin secretion during *in vivo* hyperglycemic clamp studies. Next generation mRNA sequencing studies demonstrate a time-dependent alteration in the gene expression profile of insulin-secreting islets of Langerhans in response to Cd exposure. 199 and 206 differentially expressed (DE) genes were found at 1 month and 4 months of exposure respectively. The overlap between the DE genes at these time points was only partial, suggesting time-dependent dynamic effects of Cd on islet physiology.

PS 2600 Chromate-Induced Loss of E2F1 Inhibits RAD51 Response in Homologous Recombination Repair

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Lung cancer is the leading cause of cancer death, however the mechanisms of how lung carcinogens cause cancer are poorly understood. Metals including hexavalent chromium [Cr(VI)] are known to induce chromosome instability, an early event in lung cancer. Failure of homologous recombination repair is a key mechanism of chromosome instability. We have shown particulate Cr(VI) causes DNA double strand breaks and prolonged exposure impairs the effector step of homologous recombination targeting the RAD51 protein. It is currently unknown how Cr(VI) impairs RAD51 function. This study considers the effects of particulate Cr(VI) on transcription of RAD51 specifically focusing on its primary transcription factor, E2F1. The objective of this study was to show prolonged exposure to particulate Cr(VI) inhibits RAD51 expression by affecting E2F1-mediated transcription of RAD51. We found exposure to particulate Cr(VI) reduced RAD51 and E2F1 mRNA and whole cell and nuclear protein levels. Next we showed E2F1 knockdown inhibited the RAD51 response to particulate Cr(VI) by reducing RAD51 expression and nuclear foci formation. Finally, *in vitro* Cr-binding showed Cr(III), not Cr(VI) can bind to acetylated lysine as a potential physical-chemical mechanism of Cr-inhibited function. *This work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W], the Jewish Heritage Foundation for Excellence [JPW], and the NIEHS T32 Training Grant [5T32ES011564-12 to JPW and RMS].*

PS 2601 A Mechanism Underlying Methylmercury-Induced TNF-α Expression in Mouse Brain

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Methylmercury has been known as a potent neurotoxicant, however the mechanism involved in its neurotoxicity is not fully elucidated. Recently, we found that the TNF-α might play a part in the selective damage of neurons by methylmercury in mouse brain, however the underlying mechanism of its induction is obscure. In this study we examined the responsible cells that involved in the induction of TNF-α by methylmercury to elucidate the detailed mechanism of TNF-α induction by methylmercury in mouse brain. Seven days after subcutaneous injection of C57BL/6 mice with saline or methylmercuric chloride (25 mg/kg) and TNF-α mRNA were determined in the brain by *in situ* hybridization. We found that TNF-α was hardly expressed in the saline-administered group, whereas TNF-α-expressing cells were observed in the whole brain in the methylmercury-treated group. It has been reported that astrocyte and microglia are involved in the induction of TNF-α. Next, we performed immunostaining using antibodies for GFAP or IBA1, which are specifically expressed in astrocyte and microglia, respectively. The TNF-α-expressing cells did not overlap with the GFAP-positive cells, but with the IBA1-positive cells. The results indicate that microglia are mainly involved in TNF-α induction by methylmercury in mouse brain. In addition, methylmercury induced TNF-α

expression in mouse primary microglia and mouse microglial cell line BV-2. MAP kinase pathways are known to be mainly involved in TNF-α induction in microglia. Methylmercury induced phosphorylation of JNK, p38 and ERK, the major MAP kinases, in a does dependent-manner in BV-2 cells. However, an inhibitor of JNK or ERK did not affect TNF-α induction by methylmercury, whereas p38 inhibitor significantly suppressed it. Pretreatment of actinomycin D, a transcription inhibitor, suppressed TNF-α induction by methylmercury. This indicates that several transcription factors may be involved in this induction. Therefore, we performed knockdown of NF-κB (p65) or AP-1 (cJun and/or cFos), which is a transcription factor involved in TNF-α expression and found that only the p65 knockdown partially suppressed TNF-α induction by methylmercury. These results suggest that methylmercury induces TNF-α induction via activating p38 and p65 in microglia.

PS 2602 Blood Metal Correlations in an Urban Population in Brazil

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Human biomonitoring (HB) approaches have been used to address the relationship between environmental exposures to metals, body burden, and possible adverse health effects. The body burden of metals depends on several factors, such as toxicokinetics, exposure routes and frequencies of exposure. Therefore, HB studies should also consider the possible interaction between metals given that metals share exposure sources and metabolic pathways. A cross-sectional census-based design study was conducted in 2011, enrolling 903 subjects living in an urban population in Southern Brazil. Blood levels of the arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), selenium (Se) and zinc (Zn) were quantified by ICP-MS. The aim of the study was to evaluate whether a correlation existed between blood metal levels in a group of hypertensive and non-hypertensive subjects. Using the software R, Pearson's correlation coefficients were calculated to determine correlations between these metals. The following positive correlations ($p<0.01$) were observed in the non-hypertensive group: Cu and Zn (r= 0.709), Fe and Se (r=0.507), Fe and As (r=0.605) and Mg and Fe (r=0.688). In the hypertensive group, correlations between blood Cu and Zn levels (ρ=0.665), blood Fe and As (ρ=0.630) and blood Mg and Fe levels (ρ=0.678) were observed. Few epidemiological studies have addressed and even less so accounted for the potential correlation between metals in the analysis of metal-related health effects. Here, we establish that HB of blood metal levels must take into account interactions between metals, reflecting real life exposure scenarios.

PS 2603 The Fate of Cells That Escape Cr(VI)-Induced Cell Death

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Hexavalent chromium (Cr(VI)) compounds are known human lung carcinogens; but the carcinogenic mechanism is poorly understood. Cr(VI) induces DNA damage which normally leads to apoptotic responses to avoid transformation and carcinogenesis. Evasion of apoptosis is a hallmark of carcinogenesis, but it is unknown how Cr(VI)-damaged cells are able to escape cell death. We exposed human lung cells to low concentrations of zinc chromate for 6 months (0.0125, 0.025 and 0.05 ug/cm^2). Growth parameters, and chromosome instability were measured every 10 days throughout treatment; soft agar growth was measured in the middle and at the end of treatment. Increases in chromosomal alterations were observed beginning at day 10 and increased with time, increases in numerical chromosome instability were observed before increases in structural instability. No growth in soft agar occurring during the exposure period. At the end of treatment cells were seeded at colony forming density, 10 surviving colonies for each treatment group were randomly selected, expanded into cell lines and characterized to determine permanent changes. All control clones had normal a chromosome complement. 20, 90 and 50 percent of treated clones exposed to 0.0125, 0.025 and 0.05 ug/cm^2 zinc chromate for 6 months exhibited permanent chromosome instability long after the removal of treatment. Eighty percent of abnormal clones were highly aneuploid, containing stable translocations but also many unstable numerical and structural changes. In addition 90% of the abnormal clones were able to grow in soft agar. Future work will identify chromosome specific targets and investigate how they are targeted. These data support a hypothesis that Cr(VI)-treated cells can evade apoptosis and transform into chromosomally unstable cells that continue to survive and grow. *This work was supported by NIEHS grant ES016893 (J.P.W.) and the University of Louisville School of Medicine Basic Grant Program (S.S.W).*

PS 2604 Blood Lead Levels among Auto Mechanics and Health Care Workers in the Gambia: A Case-Control Study

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Lead is one of the most widely distributed and mobilized environmental pollutants with potential for adverse health effects to humans, especially in unregulated workplaces like the auto-repair industry. In this study, we identified blood lead levels among occupationally exposed workers. The study was conducted among workers in the auto repair workers, and healthcare workers in urban Greater Banjul Metropolitan area of The Gambia. 145 auto repair workers participated as cases and 68 healthcare workers as controls. 69% of cases were between the ages of 18 and 30 years, 24.1% were between 31 to 50 years, and 6.2% were 50+ years. For the control group, 44.1% were between 18 and 30 years; 48.5% between 31 and 50 years; and 7.4% were 50+ years. The study included workers from all sections of auto repair and healthcare industries. LeadCare® II Blood Lead Testing System was used to measure blood lead levels among participants. Blood lead reference level of 5 µg/dL recommended by the CDC was adopted and blood lead levels above 5µg/dL indicated 'elevated' lead exposure. Distribution of blood lead levels by age, gender, ethnicity, marital status, use PPE amongst other variables were examined. Results showed that more than two-third of the participants 50+ years in both the case and control groups had high blood lead levels. Less than half of the participants between 18 and 30 years in the control group had high blood lead level, and more than two-third in the case group had high blood lead level. Years of work had increased odds for high blood lead levels. Workers who worked in the industry for 4 to 9 years (OR=1.45; 95% CI=0.36-5.82); and 10+ years (OR=2.69; 95% CI=0.70-10.34) had increased odds for high blood lead levels. Increased odds for high blood lead levels were found for panel beating (OR=1.49; 95% CI=0.43-5.15) and for no PPE use (OR=21.99; 95% CI=1.39-346.95). The epidemiology of blood lead levels among occupationally exposed workers in The Gambia is similar to those in other low-income countries. This study further emphasis the need to promote and legislate PPE use in occupationally exposed workers.

PS 2605 Systematic Characterization of Hexavalent Chromium and Potential Female Reproductive Outcomes: Application of US EPA Critical Appraisal Tools and Stepwise Inclusion of Mechanistic Data

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Though female reproductive toxicity has not been the primary endpoint of focus in previous risk assessments for hexavalent chromium [Cr(VI)], additional studies have been published since authoritative assessments. Herein, the objective is to: (1) build on previous assessments, including both newer studies of apical outcomes and mechanistic data, and (2) apply systematic review methods to contribute in developing best practices in systematic review. Evidence was identified using a modified search strategy from the USEPA-IRIS Cr(VI) protocol to identify studies specific to female reproductive effects, resulting in 21 potentially relevant studies in experimental animals (no human data were identified as relevant) characterizing apical outcomes. These studies were then divided into lines of evidence by endpoint (e.g., implantation loss, number of live pups). Critical appraisal, conducted on the experimental level, involves assessment of reporting quality, risk of bias (RoB), and sensitivity, results of which highlight the need for topic-specific refinements to differentiate elements of validity. An added metric in the reporting quality assessment specific to oral feeding studies was to consider the potential reduction of Cr(VI) to Cr(III) and its impact on exposure. The findings determine the need to systematically review the mechanistic data (and demonstrate a stepwise application of systematic review). Initial evaluations of the utility and feasibility of mechanistic data were also conducted based on assessment of high-throughput screening (HTS) data from the ToxCast/Tox21 screening programs. Cr(VI) was tested in 22 assays related to endocrine signaling and exhibited inconsistent results between assays. In order to integrate these data systematically, observed activity (e.g., receptor antagonism) require additional assessment, including critical appraisal and biological-pathway-based considerations (including dose) relative to the potential for adversity. This application highlights the advantages of applying systematic review techniques as part of best practices and the complexities in applying such methods in a reproducible, rigorous, and topic-specific manner to a heterogeneous data set.

2606 Rodent Hair Is a Poor Biomarker for Internal Exposure Dose of Manganese

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Manganese (Mn) is an essential metal in the human body implicated in numerous biological processes including insulin signaling and maintaining normal brain and nerve function. Currently, commonly used biological specimens as biomarkers of over-exposure to Mn include blood, urine, plasma, nails, and hair. The motivation for this study is the absence of evidence for hair as a biomarker to quantify internal *vs* external exposure. Two rodent models of C57BL/6J mice and Sprague Dawley rats were exposed in two blocks of 3 subcutaneous injections every 3 days starting on day 0 and 20. The control group received two blocks of saline (vehicle), the second treatment group received two blocks of Mn (50 mg/kg $MnCl_2 \cdot 4H_20$) and the third group received one block of Mn injections and one block of methylmercury (MeHg) injections. MeHg was used as a positive control (3mg/kg) as it has been shown to accumulate in hair upon internal exposure. Hair was collected from the animals on days 0 and 60 of the study from all treatment groups. The animals were sacrificed on day 60 of the study and tissue samples including brain, liver, and kidney were collected. Mn concentrations in the hair samples were quantified by inductively coupled plasma-mass spectrometry (ICP-MS). Methods for washing and digesting hair samples have been established in the human biomarker literature and were applied here to detect Mn using ICP-MS. Our results from the mice show no significant correlation between internal Mn dose and hair Mn content, whereas hair Hg was significantly elevated in exposed *vs* non-exposed samples ($p<0.001$). We are in process of analyzing the hair samples from rats for hair Mn content. Additionally, whole body Mn content in rats and mice is also being evaluated with *In Vitro* Neutron Activation Analysis (IVNAA). Overall, we find no evidence to support the use of hair as a valid biomarker for internal exposure to Mn at a neurotoxic level.

2607 Prolonged Particulate Hexavalent Chromium Exposure Disrupts Centrosome Regulation Proteins and Causes Centrosome Amplification

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Hexavalent chromium [Cr(VI)] is a well-known lung carcinogen with environmental and occupational exposure risks. A key mechanism for Cr(VI) carcinogenesis is induction of chromosome instability (CIN), including changes in chromosome number which can arise due to centrosome amplification. Centrosome amplification is observed in many types of cancers and is present in neoplasias, tumors, and is associated with aggressive cancers. This study examines centrosome amplification in Cr(VI)-exposed human lung cells and human lung tissue, and investigates the mechanism of Cr(VI)-induced centrosome disruption in human lung cells. Our hypothesis is Cr(VI) disrupts proteins that regulate centrosome duplication, leading to centriole disengagement and centrosome amplification. Centrosome amplification was measured by fluorescent immunostaining of gamma-tubulin, a centrosome marker, in lung tumor tissue from chromate workers and in Cr(VI)-exposed human lung cells. In Cr(VI)-exposed human lung cells, centriole disengagement was analyzed by fluorescent immunostaining of centrioles with CNAP1 and centrin, markers for the proximal and distal centriole ends. Protein extracted from Cr(VI)-exposed human lung cells was analyzed by western blot. During interphase in normal cells, centriole engagement blocks premature centrosome duplication. Our findings show increasing concentrations and prolonged exposure to Cr(VI) increased centriole disengagement in human lung interphase cells. We also measured decreased protein levels of the separase inhibitors, securin and cyclin B1, which are key to regulating the centrosome cycle. Supernumerary centrosomes were observed in human lung tumor tissue from chromate workers. Together, these data demonstrate prolonged Cr(VI) exposure induces centriole disengagement, centrosome amplification, and causes decreased levels of proteins that inhibit centriole disengagement. Ongoing studies using securin and separase siRNA knockdown are elucidating the role of these proteins in the Cr(VI) carcinogenic mechanism. *This work was supported by NIEHS grant R01ES016893 (J.P.W.) and T32-ES011564 (J.H.T.).*

2608 Formation of Plumbojarosite (PLJ) Reduces Bioavailability of Soil-Borne Lead (Pb)

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Exposure to Pb during early life has long-lasting adverse effects on health. Ingestion of Pb-contaminated soil is a major route for exposure of children to this toxic metal. Soil remediation procedures that alter physiochemical properties of soil-borne Pb can limit exposure by reducing gastrointestinal Pb uptake. A novel approach for remediation of soil Pb uses addition of iron (Fe) sulfate and application of heat to promote formation of PLJ, a poorly soluble Pb-Fe sulfate compound. Here, two Pb-contaminated soils and samples of a low-lead soil spiked with various Pb compounds (i.e., carbonate, chloride, phosphate, or sulfate) were treated to convert native Pb species to PLJ. We used a mouse assay to examine tissue Pb distribution after ingestion of diets amended with untreated or treated soils. Bone and blood Pb levels were determined to evaluate uptake across the gastrointestinal barrier. For both Pb-contaminated soils and all Pb compounds, bone and blood Pb levels were significantly lower ($P<0.001$, student t-test) in mice that consumed diets amended with treated soils than in mice that consumed diets amended with untreated soils. After treatment, estimated relative bioavailability (RBA) of Pb in both soils and for all Pb compounds were reduced by more than 90% compared to RBA estimates for untreated soils or compounds. X-ray absorption spectroscopy was used to determine Pb species in soil-amended diets and in feces excreted by mice consuming these diets. Treatment of Pb-contaminated soils or Pb compounds consistently converted more than 90% of all Pb species in these materials to PLJ. Speciation of Pb in feces from mice fed diets containing soils or Pb compounds treated to promote PLJ formation found no evidence that ingested PLJ underwent chemical transformation during transit of the gastrointestinal tract. This evidence suggests that formation of PLJ could be an effective strategy to reduce the RBA of Pb in soil and minimize this medium's role as a source of exposure to Pb in young children. *This abstract does not represent US Environmental Protection Agency policy.*

2609 Mechanism of Cadmium Carcinogenesis—Epigenetic Modification and the Induction of CSC-Like Cells

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Cadmium (Cd) is a ubiquitous pollutant in the environment and a known carcinogen causing lung cancer. However, the mechanism of Cd carcinogenesis has not been well defined. Cd is considered a weak mutagen and has been suggested that it causes non-genotoxic effects such as epigenetic modifications. The goal of this study is to investigate the role and mechanism of epigenetic deregulations in Cd exposure-induced cell transformation, cancer stem cell (CSC)-like cell property and tumorigenesis. After 9 months of Cd exposure, malignant transformation of immortalized human bronchial epithelial cells (BEAS-2B) was evidenced by increased formation of colonies in soft agar, suspension culture spheres and tumor formation in nude mice. Suspension sphere formation assay demonstrated that Cd-transformed cells display CSC-like property, which was further evidenced by increased aldehyde dehydrogenase (ALDH) activity, up-regulated CD133 expression level, as well as increased expression of several stemness-related proteins, including Nanog, Oct4, Klf4, Klf5, and c-Myc. That only CD133-positive population capable of forming spheroids suggests that CD133 could be a cell surface marker of the induced CSC-like population. Increased levels of DNA and histone methyltransferases, as well as elevated levels of histone 3 lysine 9 and 27 methylation indicate that chronic Cd exposure causes epigenetic dysregulations. In addition, several oncogenic long non-coding RNAs (lncNRAs), such as DUXAP10 and HOTAIRM1, were found up-regulated in Cd-transformed cells. Moreover, knockdown of DUXAP10 leads to decreased expression of stemness-related genes and reduced number of suspension culture spheres. In conclusion, long term Cd exposure causes cell malignant transformation. The transformed cells displayed CSC-like property. Epigenetic dysregulations may contribute to Cd-induced CSC-like property and cell transformation.

2610 Loss-of-Function Variant in the BCRP/*ABCG2* Transporter Increases Cadmium Renal Injury *In Vitro*

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Cadmium is a high priority environmental pollutant present in tobacco smoke and contaminated foods. With chronic exposure, cadmium accumulates in multiple tissues including the kidneys where it causes toxicity. The breast cancer resistance protein (BCRP, *ABCG2*) is an efflux transporter in kidney tubules that facilitates the urinary secretion of drugs and toxins. Our previous studies indicate that the *ABCG2* genetic variant Q141K (C421A) reduces transport of BCRP substrates due to altered membrane trafficking. In the current study, we sought to 1) assess the *in vitro* ability of BCRP to efflux cadmium and protect kidney cells from injury and 2) determine whether this protection is disrupted by the Q141K variant. Cadmium ($CdCl_2$) accumulation and cellular stress and toxicity were assessed in human embryonic kidney 293 (HEK293) cells stably expressing plasmids containing an empty vector (EV), BCRP wild-type (WT), or BCRP variant (Q141K) gene. Intracellular $CdCl_2$ accumulation was significantly higher in EV cells compared to BCRP WT cells, confirming that cadmium is a novel substrate of BCRP. Following exposure to $CdCl_2$ (2.5 to 10 μM) for 48 h, greater apoptosis (100-300%) was observed in EV cells compared to WT BCRP cells. Exposure to $CdCl_2$ (0.5 and 1 μM) induced mRNA and protein expression of stress-related genes including metallothionein 1A and 2A (MT-1A and 2A), NAD(P)H quinone dehydrogenase 1 (Nqo1), and heme oxygenase (HO-1) to a greater extent in EV cells compared to WT BCRP cells. While the BCRP Q141K variant protected against $CdCl_2$-induced activation of stress pathways and cytotoxicity compared to EV cells, the extent of protection was less than that observed with WT BCRP. In conclusion, the BCRP/*ABCG2* transporter protects against cadmium toxicity through active efflux from kidney cells, a response that is limited by the loss-of-function Q141K genetic variant. *Supported by R01ES029275 and P30ES005022.*

2611 Trophic-Level Interactive Effects of Dietary Nutrients (Phosphorus and Nitrogen) on the Toxicities of Cadmium, Arsenic, and Their Binary Mixtures

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Various anthropogenic activities alter the concentrations of dietary nutrients in environmental media. These stoichiometric changes may interact with the uptake and effects of metals in exposed organisms. It is therefore important to assess trophic-level interactive effects of the changes in media and dietary nutrients on the toxicities of metals; to provide nutrient-mediated risk factors of toxic metals exposures in environmental media. This study investigates the single and joint toxicities of cadmium (Cd) and arsenic (As) in algae, *Scenedesmus acutus* and *Daphnia pulex*, under varied media and dietary phosphorus (P) and nitrogen (N) conditions. Acute studies were conducted to assess the IC_{50}s (7 d) and LC_{50}s (24, 48, 72, and 96 h) of the metals (Cd and As) against *S. acutus* and *D. pulex*, respectively, when acting singly and as a binary mixture. Algae growth rate and the 7-d IC_{50}s were determined under low (20% P, 25% N), median (60% P, 62.5% N) and optimum (100% P, 100% N) dietary nutrients of a COMBO media. Chronic studies (14 d – 21 d) were conducted to assess the developmental (survival, growth), reproductive (brood size, timeline), behavioral (distance moved, velocity, acceleration) and physiological (feeding, heartrate, respiration) responses of *D. pulex* exposed to Cd (0, 18.75, 37.5 and 75.0 μg/L) and As (0, 625, 1250 and 2500 μg/L) under low, median and optimum media and dietary P and N conditions. Results showed enhancement of Cd and As toxicities (singly and mixture) against *S. acutus* and *D. pulex* cultured under low P and low N conditions. There was a concentration-dependent decrease in *S. acutus* growth rate with IC_{50}s in the order Cd+As > Cd > As in Low P > Low N > COMBO media with evidences of hormesis. Furthermore, binary mixtures of Cd and As against *D. pulex* were additive (24 h) and synergistic (> 24 h) with significant interaction ($p<0.05$) effects of media nutrients, Cd and As on algae growth, and survival, reproduction, physiology and behavior of *D. pulex*. The importance of taking into account the interactive effects of contaminant mixtures and dietary nutrients in environmental media on toxicity outcomes for effective risk assessment was discussed.

2612 Understanding the Role of the Manganese Transporter Slc30a10 in Developing Mice

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Manganese (Mn) is an essential, but potentially toxic trace metal acquired through the diet. Mn excess leads to a Parkinson's-like disorder which is most commonly seen in industrial workers. Young individuals are believed to be particularly susceptible to the irreversible damages of Mn excess due to poor development of regulatory absorption and excretion pathways. Therefore it is important to better understand early mechanisms of Mn metabolism to prevent Mn toxicity. In 2012, the first case of inherited Mn excess was identified. Patients exhibited systemic Mn excess, liver cirrhosis, a Parkinson's-like disorder, and polycythemia (increased red blood cell counts). Mutations were identified in SLC30A10. In this study, we hypothesize that SLC30A10 plays a key role in Mn homeostasis during development. To address this, we generated a global Slc30a10-deficient (*Slc30a10$^{KO/KO}$*) mouse line. *Slc30a10$^{KO/KO}$* mice are smaller than wild-type littermates. Postnatal day (P) 14 *Slc30a10$^{KO/KO}$* mice exhibit comparable Mn levels as *Slc30a10$^{+/+}$* mice. However, by P21 *Slc30a10$^{KO/KO}$* mice exhibit a 17-fold increase in liver Mn levels. Interestingly Mn levels in *Slc30a10$^{KO/KO}$* mice decrease in the liver by P28, suggesting the development of other Mn excretion pathways. To evaluate the development of Mn excretion in developing mice, a radioactive approach was utilized. In this experiment, ^{54}Mn is injected intraperitoneally at P14. Whole body counts are then recorded every other day until the time of harvest. While *Slc30a10$^{KO/KO}$* mice develop severe Mn excess, the development of the capacity to excrete Mn is only delayed, not abolished, in these mice. This suggests that factors other than Slc30a10 play key roles in the development of Mn excretion. We hypothesize that the development of Mn excretion is both Slc30a10- and Mn-dependent. When *Slc30a10$^{KO/KO}$* mice are raised on a Mn-deficient diet, the development of Mn excretion is further delayed despite attenuated tissue Mn excess. We speculate that Slc30a10-independent mechanisms of excretion are induced when tissue Mn levels exceed a threshold, but this threshold decreases with age. We predict developing mice require a higher threshold of Mn to develop the ability to excrete Mn when compared to older mice. Overall, this study establishes early mechanisms of Mn regulation and suggests Slc30a10 plays a critical role in preventing Mn toxicity during development.

2613 Cellular and Molecular Mechanisms Underlying Arsenic-Associated Dysregulation of the Glucocorticoid Receptor (GR) Signaling Pathway in the Placenta

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In utero exposure to inorganic arsenic (iAs) is linked to many early and later life adverse health effects. The development of these health outcomes may be related to altered function of nuclear transcription factors in the placenta, such as the glucocorticoid receptor (GR). We have previously shown that placental exposures to environmentally-relevant levels of iAs are associated with a dysregulation in placental mRNA expression and DNA methylation of GR-associated genes that play a role in fetal growth and development. Of relevance to iAs-associated health outcomes is the metabolism of iAs into its monomethylated (MMAs) and dimethylated metabolites (DMAs), which have different toxicological endpoints compared to iAs. Given this, we investigated cellular mechanisms that underlie observed modulation of the placental GR signaling pathway, and to determine if iAs metabolites alter expression of GR genes. JEG-3 cytotrophoblasts were treated with non-toxic doses of iAs^{III} (0.5-3 μM) and MMA^{III} or DMA^{III} (0.05-0.25μM) for 24 hours. To examine if GR signaling cellular processes may be dysregulated by iAs exposure, we evaluated GR activation and nuclear translocation, among others in the iAs treated samples. A reduction in nuclear translocation of the GR was observed following iAs exposure, which may drive the observed repression of GR-associated mRNA expression at higher doses of iAs. A biphasic dose-response was observed with GR activation following iAs treatment, with low doses hyperactivating GR, and high doses reducing GR activity. These data are important as they coincide with previously observed biphasic responses in GR-associated gene expression following iAs treatment. MMA^{III} and DMA^{III} were found to be associated with alterations in eight GR-associated genes including metallothionein 2A (*MT2A)*, interleukin 6 Receptor (*IL6R),* and TNF alpha induced rotein *(TNFAIP)*, which play a role in placental pathophysiology and fetal development. This study builds on previous evidence from our lab that iAs disrupts GR signaling in the placenta, provides a novel cellular mechanism that may underlie iAs alterations in placental GR signaling, and demonstrates a relationship between iAs metabolite exposure and genomic modulation of the GR-pathway in the placenta.

2614 Arsenic and Benzo(a)pyrene Co-exposure-Transformed Cells Display Apoptosis Resistance by Upregulating Mcl-1 Expression Level through Synergistically Activating the PI3K/Akt Pathway

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Both arsenic (As) and benzo(a)pyrene (BaP) are known human carcinogens causing lung cancer. Human exposure to As and BaP is common and co-exposure to As and BaP could cause more than an additive effect than exposure to As or BaP alone. Our recent study showed that As and BaP co-exposure caused a synergistic effect in inducing cell malignant transformation, cancer stem cell (CSC)-like property and tumorigenesis. However, the underlying mechanism remains largely unknown. Evading apoptosis is one of well-established hallmarks of cancer. The goal of this study was to investigate whether As and BaP co-exposure-transformed cells display apoptosis resistance, which contributes to their increased malignant property. It was found that As and BaP co-exposure transformed cells (BEAS-2B-As+BaP) exhibit a significantly stronger resistance to apoptosis induced by treatment with ABT-737, etoposide, cisplatin or doxorubicin. Mechanistic studies revealed that BEAS-2B-As+BaP cells have a significantly higher expression level of the anti-apoptotic protein Mcl-1 than that of cells transformed by exposure to As (BEAS-2B-As) or BaP (BEAS-2B-BaP) alone. Stably knockdown Mcl-1 greatly reduced apoptosis resistance of BEAS-2B-As+BaP cells to ABT-737 treatment. Mcl-1 knockdown in BEAS-2B-As+BaP cells also significantly reduced their CSC-like property. Further mechanistic studies revealed that As and BaP co-exposure increases Mcl-1 level by synergistically activating the PI3K/Akt pathway. Pharmacological inhibition of PI3K/Akt significantly reduced Mcl-1 level in BEAS-2B-As+BaP cells and decreased their resistance to apoptosis induced by ABT-737 treatment. In contrast, stably expressing a constitutively active Akt (myr-Akt) reversed the effect of PI3K inhibition on Mcl-1 level and apoptosis resistance. It was further determined that PI3K/Akt pathway activation up-regulates Mcl-1 level in BEAS-2B-As+BaP cells by increasing Mcl-1 protein stability through reducing Mcl-1 ubiquitination. Together, these findings suggest that As and BaP co-exposure-transformed cells display a stronger resistance to apoptotic cells death, which may contribute significantly to the synergistic carcinogenic effect of As and BaP co-exposure.

2615 Alteration of *Drosophila* Nrf2 Signaling in Developing Neuromuscular Compartments Mediates Methylmercury Impacts on a Neuromotor Behavior

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Methylmercury (MeHg) is a developmental toxicant. Exposure to MeHg *in utero* can lead to neurocognitive and neuromotor deficits in children. The presentation of neuromotor deficits suggests that MeHg may contemporaneously target developing neurons and muscles. Induction of oxidative stress is an established mechanism of MeHg toxicity, in which the Nrf2 antioxidant pathway is a recognized protective mechanism. Tissue-specific investigations into the role of Nrf2 moderating MeHg-induced oxidative stress have been limited to cell culture, making it difficult to pair findings with a neuromuscular output. *Drosophila melanogaster* contains a homologous inducible antioxidant pathway, whereby the *Drosophila* CncC protein is the ortholog to the mammalian Nrf2 protein. Nrf2 and CncC are both negatively regulated by Keap1. Our lab has established eclosion, a neuromuscular behavior required for adult *Drosophila* emergence from the pupal case, as a sensitive functional output of MeHg toxicity. Developmental exposure to MeHg induces a "balling" or myosphere phenotype in the developing indirect flight muscles (IFMs). We hypothesize that increased CncC signaling across various developmental windows and neuromuscular compartments will enhance eclosion ability and protect muscle development by reducing MeHg-induced oxidative stress. Utilizing the GAL4/UAS transgene technique, an increase in CncC signaling via CncC overexpression or knockdown of Keap1 exclusively in developing CNS neurons, motor neurons, or musculature rescues eclosion ability upon MeHg exposure. With epifluorescence imaging, we observe a decrease in myosphere numbers in the developing IFMs with increased CncC signaling in muscles or neurons upon MeHg exposure. Measuring relative ROS levels, through the DCF-DA assay, indicated that a tissue-specific increase in CncC signaling decreases MeHg-induced ROS, suggesting rescue in eclosion and IFM development by increased CncC signaling occurs via reducing MeHg-induced ROS. These findings support the notion that sensitive non-neuronal target tissues of MeHg toxicity exist, providing evidence that neuromotor deficits seen in children with *in utero* MeHg exposure could be caused by disruption in both neurogenic and myogenic processes. Future research will be aimed at understanding myogenesis as a target in MeHg-mediated neuromuscular behavioral deficits. *Supported by R01ES025721 and T32ES007026.*

2616 Using Gene Expression Analysis to Support Risk Assessment of Metal Mixtures in Freshwater Systems

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Mining waste can impact ecosystems worldwide, and the associated releases of potentially toxic metals may threaten human and environmental health. Current regulation of metal-impacted freshwaters is informed using bioavailability models such as the Biotic Ligand Model (BLM). The BLM predicts toxicity based on water chemistry at an impacted location and on the consequent inferred accumulation of metals in the organism. Typically, these models assume additive toxicity in metal mixtures, meaning the total toxic effect is approximately a linear combination of the toxicities of each metal when acting alone. In previous work, we demonstrated that at median concentrations of both metals, some mixtures of Cd and Zn and some mixtures of Cd and Ni result in less-than-additive, acute toxicity (i.e., a protective effect) to *Daphnia magna*, an aquatic invertebrate. We also demonstrated, using RNA-seq and inductively coupled plasma - mass spectrometry (ICP-MS) that the protective (less-than-additive) effect observed in Cd-Zn mixtures does not appear to be fully explained by simple competition between the two metals for binding to a biotic ligand. RNA-seq data suggests that a mechanistic physiological response contributes to the Cd-Zn mixture effects. *Daphnia* exposed to moderately toxic doses of Cd alone differentially expressed over 1000 genes (DEGs) at False Discovery Rate (FDR) <0.1. Interestingly, in the Cd-Zn mixture at the same Cd concentration, the number of DEGs decreased to 291. Of the 291 DEGs, 282 were shared between Cd and the Cd-Zn mixture. Exposure to Zn alone at the same Zn concentration as in the mixture resulted in only 24 DEGs. We suggest that these finding are not consistent solely with metal-metal binding competition at a biotic ligand. Biological-pathway and cellular-process analyses support an organismal physiological response as a key role in the less-than-additive (protective) effects. Diverse cellular responses were identified in the Cd-only exposure that are consistent with the observed toxicity, including induction of the unfolded protein response (UPR), antioxidant responses such as induction of glutathione biosynthesis, glutamate-mediated signaling, and down regulation of genes related to digestive proteolytic enzymes. Interestingly, several key responses appear to be mitigated in the mixture treatment group. Specifically, the induction of the unfolded protein response and the glutamate-mediated signaling were not observed in Cd-Zn mixture. Some biological effects, such as the induction of glutathione, are present in both the Cd-only treatment and the mixture treatment. These finding are consistent with a model in which Cd is still bioavailable in the mixture, but Zn may prevent deleterious physiological effects. An enhanced understanding of the interaction between Cd and Zn (as well as Cd and Ni) will allow development of more accurate models of metal-mixture toxicity for use in risk assessments. Overall, this work indicates an important role for an internal physiological interaction in the presence of metal mixtures. More work is required to better understand that role.

2617 Selenium Protection against Inorganic Mercury-Induced Cytotoxicity via Modulating Oxidative Stress in PC12 Cells

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Mercury (Hg) in its all forms, including inorganic Hg (iHg) is an environmental contaminant that could induce toxicity and diseases in human. However, a little has been known about the underlying mechanisms responsible for iHg toxicity. Selenium (Se) is an essential trace element, recognized as an antioxidant and protective agent against metal toxicity. The purpose of this research was to elucidate the molecular mechanism of iHg induced toxicity as well as, to investigate the ameliorative effects of Se against iHg induced toxicity in PC12 cells. Cytotoxic assays have been shown that iHg (5 μM) induced oxidative stress and intrinsic apoptotic cell death *via* oxidizing glutathione, damaging DNA, degrading cell membrane integrity, down-regulating mTOR, p-mTOR, akt, ERK1 and caspase 3, and up-regulating cleaved caspase 3 and cytochrome c release in PC12 cells upon 48h of exposure time. Moreover, flow cytometry analysis confirmed iHg induced apoptosis in the cells. Again, co-treatment of Se (5 μM) appeared to inhibit intrinsic apoptosis and oxidative stress induced by iHg (5 μM) *via* boosting GPx amount, improving glutathione content, limiting DNA degradation, improving cell membrane integrity, up-regulating mTOR, p-mTOR, Akt, ERK1and caspase 3, and down-regulating cleaved caspase 3 and cytochrome c leakage in PC12 cells. In addition, flow cytometry analysis confirmed Se-protection of cells from iHg induced apoptosis. The contribution of nrf2 and HO-1 activation was not significant in iHg-toxicity or Se-protection mechanisms in PC12 cells. In conclusion, these results suggested that glutathione level decrease plays a critical role in iHg induced oxidative stress and co-treatment of Se attenuates iHg-cytotoxicity through its antioxidant properties.

2618 Manganese-Induced Parkinsonism Is Not Associated with Dopamine Neuron Loss in the Midbrain of Nonhuman Primates or SLC39A14 Knockout Mice

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Humans with chronic exposure to high levels of Mn exhibit parkinsonism with dystonia that is refractory to L-dopa, an effective therapy used in idiopathic Parkinson's disease (iPD). iPD is characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies whereas Mn-induced neurotoxicity results in cell loss and gliosis in the globus pallidus, inhibition of striatal dopamine release, and an apparent absence of Lewy bodies and DA neuron loss in the SNpc. A major limitation of prior neuropathological investigations in animal models of Mn-induced parkinsonism was the lack of unbiased stereological quantification of DA neuron numbers in the SNpc. Here, Mn-exposed non-human primates and a genetic mouse model of Mn-induced parkinsonism were used to ascertain if neurotoxic brain levels of Mn impacted dopamine neuron density (TH Nv) or soma volume in the SNpc. Adult male cynomolgus macaques (ages 6-11 years) were given saline (n = 15) or 50 mg/mL of MnSO4 monohydrate (n = 8) intravenously twice per week for a minimum of one year. Several animals also received amphetamine (AMPH, 2 mg/kg) during PET scans to measure dopamine release in the frontal cortex. TH Nv was quantified for the entire SNpc in macaques and mice, as well as in the dorsomedial (DM) and ventrolateral (VL) subfields in macaques. Mn exposure was not associated with DA neuron loss in the SNpc regardless of AMPH exposure in macaques (p's ≥ 0.19) or in adult *SLC39A14*-KO male ($p = 0.96$) compared to control macaques or WT adult mice. Mn treatment in macaques was correlated with significantly decreased VL SNc TH soma volume ($p = 0.02$), and Mn-treated macaques with AMPH exposure for PET imaging had greater DM and total SNc TH Nv compared to both PET-imaged controls and non-imaged Mn-treated animals (p's = 0.01). Furthermore, the motor function deficits that were observed in the SLC39A14-KO mice were not associated with striatal dopamine depletion. These data support previous findings in humans that Mn exposure spares the DA system in the SNpc and that Mn-induced parkinsonism and iPD are distinct diseases with different neuropathological etiologies. *Funding: NIH R01ES010975, TRG.*

2619 Trace Element and Blood Film Biomonitoring in Adolescents from Fishing Communities at the Caribbean

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Human activities performed with inadequate regulation and supervision, increase the load of trace elements in the environment resulting in pollution. This situation is a growing problem in public health since there is a continuous exposure to these chemicals in people who live near to industrial areas, in particular, for some vulnerable groups, especially adolescents, due to their stage of development. Analysis of these elements in human hair has been reported as an indicator of regional geochemistry and health status. The aim of this study was to evaluate the relationships between hair trace element levels and the different blood cell types in adolescents living on Tierrabomba Island, Colombia, located near an industrial area. Individuals from San Onofre, a non-industrial site, were used as reference. Hair samples were collected to quantify trace metals by ICP-MS and peripheral blood samples to perform blood film analysis. Boron, arsenic, cadmium and tungsten levels were greater in Tierrabomba Island. Scandium, cobalt, zinc, yttrium, tin and barium concentrations were superior in the reference site. Statistical differences between studied groups were registered for several blood smear parameters, such as lymphocyte, eosinophil and monocyte percentages, as well as for hypochromia and anisocytosis. Barium content was associated with fish consumption. A significant positive correlation was found for scandium and neutrophils ($r=0.156$, $p=0.017$), but it was negative for lymphocytes and platelets. Cadmium, vanadium, nickel, molybdenum, copper, tin, barium, tungsten and lead correlated negatively with platelets. Arsenic was positively linked to eosinophils ($r=0.130$, $p=0.047$). Interestingly, yttrium correlated with micronucleus frequency ($r=0.148$, $p=0.024$). These results indicate exposure to trace elements is a risk factor for the blood system health in adolescents from fishing communities. *Colciencias-University of Cartagena (Grant 110777757883, 778/2017; 727-2015).*

2620 Kiwifruit Peel Beads for Removing Heavy Metals from Drinking Water by Biosorption

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Contamination of drinking water with heavy metals is a serious concern and sustainable techniques are being developed to cater to communities across the globe for the low-cost removal of these toxicants. Biosorption is one such method because of its advantage of being economical and generating non-toxic sludge. In this study, chemically unmodified kiwifruit peels (KP) were immobilised on sodium alginate as beads and used to study the biosorption removal of arsenic (As V) and six heavy metals, namely, cadmium (Cd II), chromium (Cr VI), copper (Cu II), mercury (Hg II), lead (Pb II) and nickel (Ni II) from a cocktail solution at low concentration (0.1 mg L-1) and neutral pH conditions simulating that of drinking water. Results showed that KP bead biosorbed Cd, Cu, Ni, Pb, Hg, Cr and As ions with approximately 92%, 84%, 75%, 67%, 56%, 34% and 17% biosorption, respectively at equilibrium and neutral pH. Kinetic data were modelled by standard biosorption models such as film diffusion (FD), pore diffusion (PD), pseudo-first order (PFO), pseudo-second order (PSO) and Elovich equation. The rate of diffusion and surface attachment was the fastest for Hg followed by Cd and Ni ions. Elovich Equation was the least fitting model suggesting that the biosorption of ions by KP bead was not by chemisorption. Thermodynamic studies revealed that the biosorption of ions by KP bead were endothermic reactions that were spontaneous and physical in nature. KP beads can, therefore, be used as effective biosorbents for removing heavy metals from drinking water.

2621 Synthesis and Structural Analysis on Ionic Triphenyltin Chloride Complexes with Oxalic Acid

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There is a large need for the development of novel metal-based anticancer agents due to the low solubility and high organ toxicity of metal-based compounds such as cisplatin and its analogues. Triorganotin derivatives have been the interest of many chemists due to their biological properties against bacteria, fungus, and cancer cell lines. However, their applications are limited due to their poor water solubility. On the other hand, ionic triorganotin complexes may have improved solubilities due to their partially ionic characteristics. Our hypothesis is that ionic triorganotin derivatives will have better solubility and better activity as potential anticancer agents with reduced toxicity. Therefore, the goal of the research is to synthesize triphenyltin complexes with ionic characteristics. Syntheses of the ionic triphenyltin complex involved the reaction of a diprotic carboxylic acid, with triphenyltin hydroxide or chloride in the presence of di-ethylamine. IR/NMR spectroscopy and X-Ray Crystallography studies showed that an ionic triphenyltin complex was successfully obtained in both reactions. The ionic complex consists of a triorganotin anionic moiety, and a diethylammonium as the counterion. The anionic triphenyltin moieties has a distorted cis-trigonal bipyramid (TBP) geometry with two carbon and one oxygen atom occupying the equatorial positions and an oxygen atom and phenyl group occupying the axial positions. The ionic complex is essentially a dimer through extensive hydrogen-bonding network between the carboxylate groups (OCO) and the N atom from the ammonium cation. The results indicate ionic triphenyltin complexes can be successfully obtained in a substitution reaction of either triphenyltin chloride or hydroxide with a diprotic oxalic acid in the presence of an organic amine. Future studies will be focused on collecting data on its aqueous solubility and biological activity.

2622 Overexpression of hsa-miR-186 Induces Anchorage-Independent Growth and Chromosomal Alterations in Arsenic-Exposed Human Keratinocytes: A Preliminary Study

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Chronic arsenic (As) exposure through drinking water is a global health issue, affecting >200 million people. As is a group I human carcinogen and a clastogen causing chromosomal instability (CIN). Skin is the primary target organ for As toxicity. miRNA dysregulation and CIN are suggested mechanisms of As carcinogenesis. hsa-miR-186 is overexpressed in As-induced squamous cell carcinoma relative to premalignant hyperkeratosis. Predicted targets of miR-186 are cell cycle proteins, thus overexpression of miR-186 likely leads to CIN

and aneuploidy, which are features of cancer. We showed that overexpressing miR-186 in HaCaT cells increased numerical and structural chromosomal abnormalities. miR-186 overexpression drives malignant transformation of HaCaT cells by induction of CIN and potentially accelerates As-induced transformation. Stable clones of HaCaT transfected with miR-186 expression vector or empty vector were maintained under puromycin selection. Selected clones exposed to 0 or 100 nM As, were cultured for 10 weeks. Anchorage independent growth was tested in soft agar colony formation assay. Giemsa banding was used to produce karyotypes and cytogenetic analysis for CIN was performed. HaCaT overexpressing miR-186 and exposed to As exhibited signs of anchorage independent growth since single cells formed clusters of cells in agar while HaCaT transfected with vector control did not. As-exposed HaCaT that overexpress miR-186 also contained additional chromosomal material in one of the four copies of chromosomes 1 and 11; increases in double minute chromosomes; two copies of the abnormal isochromosome 9q and one extra chromosome 18. HaCaT authenticity was validated by the presence of reported marker chromosomes and STR mapping. Consistent chromosomal abnormalities and increased growth ability in agar were observed in As exposed miR-186 overexpressing keratinocytes, suggesting contribution of CIN to malignant transformation. Only one clone was tested per group and these karyotypic changes might be that clone's feature and not an effect of As toxicity. Thus, future research using additional clones is warranted.

2623 Impact of Arsenic Exposure on Wound Healing in Down Syndrome

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Down syndrome (DS) is characterized by a complex phenotype including intellectual disability, developmental complications, and an increased risk of chronic health conditions like congenital heart defects and early onset Alzheimer's disease. Complications with tissue regeneration - synonymous with wound healing - have also been reported in DS. It is important to note that tissue regeneration processes are similar regardless of etiology of the wound. These observations and reports of impaired wound healing with age strengthens the use of DS has a model for accelerated or atypical aging. The main aim of the current study was to characterize the wound healing response in normal and trisomy 21 cells, and to investigate the Gene x Environment interaction between DS and the ubiquitous environmental contaminant, arsenic (As). Arsenic is a naturally occurring metalloid, common groundwater contaminant, and exposure has been reported to alter wound healing processes. Here we show that DS fibroblasts, in response to a scratch wound, took significantly longer to complete wound closure, and released substantially less PGE_2 compared to euploid controls. Additionally, after exposure to environmentally-relevant concentrations of sodium arsenite, we found that As had a greater impact on cell proliferation and necrosis in trisomy 21 cells. We also found that DS fibroblasts have decreased basal gene expression of COX-1 and COX-2 and increased basal gene expression of PTGES. These are key arachidonic acid metabolizing genes, with alterations having potential to impair wound healing. Furthermore, young DS fibroblasts show decreased basal protein abundance of COX-1 and COX-2 that does not recover to control basal levels following a mechanical scratch. Our results demonstrate DS-mediated alterations in the tissue regeneration process, that can be negatively impacted by As exposure. Due to a possible lifelong disability of dysfunctional wound healing, the involvement of tissue regeneration mechanisms in various biological systems, and the passive nature by which individuals are exposed to As, these data can be applied to developmental toxicology, aging, regenerative medicine, and ultimately the treatment of those affected and not affected by trisomy 21.

2624 Mutational Processes of Arsenic and UV Co-exposure on Skin Cancer Genomes

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Arsenic is a human co-carcinogen at environmental levels. Experimental and epidemiological evidence indicates that low levels of arsenic in combination with other environmental insults such as ultraviolet radiation (UV) increases skin carcinogenesis. However, little is known about the mutagenetic processes of arsenic and UV co-exposure. Recent advances in next generation sequencing (NGS) and computational biology introduced powerful tools to analyze signatures of mutational processes. In this work, we extracted, identified, and characterized specific mutational signatures of arsenic exposure and arsenic-UV co-exposure in normal human keratinocytes using single cell whole genome sequencing. Arsenic-UV co-exposure signature differs from either the UV signature or arsenic signature, suggesting co-exposure has unique mutational process versus single exposures. Analyzing single nucleotide variation (SNVs) in whole genome sequencing results from 368 skin cancer patients from the TCGA database suggested over 40% of the cases have mutational signatures of arsenic-UV co-exposure compared to 4% of cases for the arsenic signature. In contrast, there was no evidence for UV or arsenic-UV co-exposure signatures based on SNVs from 396 bladder cancer patients. These results not only defined an arsenic-induced mutational signature for the first time, but also demonstrated the mutational signature of arsenic-UV co-exposure and in skin cancers.

2625 Decreased Expression of Schlafen 12 in Arsenic- and Cadmium-Induced Urothelial Cancer

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Arsenic and cadmium (Cd^{2+}) are environmental carcinogens associated with the development of urothelial cancer. Previous studies have shown that both arsenite (As^{3+}) and Cd^{2+} can transform the normal immortalized urothelial cell line UROtsa, into malignant lines that form tumors in immune compromised mice. These tumors resemble urothelial carcinomas with focal areas of squamous differentiation. The Schlafen family of proteins consists of short, intermediate, and long isoforms of structurally related proteins. Schalfen 12 (SLFN12) is an intermediate length member (67 kDa) expressed in human tissues with no known function. Overexpression of SLFN12 has been previously associated with increased expression of dipeptidyl-peptidase 4, E-cadherin expression, and epithelial differentiation in prostate cell lines suggesting that it is associated with a less aggressive type of a cancer. Since SLFN12 is associated with differentiation of tumors, we were interested in determining if SLFN12 is expressed in the As^{3+} and Cd^{2+}-transformed cell lines and their corresponding tumor heterotransplants along with other human bladder cancer cell lines. Our data shows that in the transformed cell lines, there was a decrease in expression of SLFN12 when compared to the non-transformed UROtsa cells. The expression was increased in the tumors produced by these transformed cells, however the staining was restricted to the more undifferentiated areas of the tumors compared to the well differentiated centers of the tumor nests. In commercially available bladder cancer cell lines RT4, HT 1197, HT 1376, T24/83, and UM-UC-3, there was reduced expression of SLFN12 when compared to the UROtsa parent cells. In human bladder cancers the expression of SLFN12 was localized to areas that were poorly differentiated. In conclusion, our study shows that the SLFN12 is expressed in the normal undifferentiated basal layer of the urothelium and its expression is restricted to the poorly differentiated areas of bladder cancers thus suggesting a role of SLFN12 in differentiation of bladder cancers.

2626 Arsenic Exposure Impairs Intestinal Cell Differentiation in Adult Mice

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Arsenic (As) is a toxicant found in drinking water and food, that is known to impair cell fate determination and differentiation in stem cells, neurons, and muscles. The intestines are a highly proliferative tissue, whose epithelial cells turn over every 3-7 days to compensate for constant abrasion. The supply of cells is maintained by resident stem cells that differentiate to form multiple types of intestinal epithelial cells. Even though the primary route of arsenic exposure is via ingestion, very little is known about its effects on intestinal cell proliferation and differentiation. Therefore, the purpose of this study is to investigate the effects of arsenic on intestinal proliferation and differentiation. Adult mice were exposed to 0 or 100ppb of arsenite in drinking water for 5 weeks, and intestinal tissue collected. To assess whether arsenic exposure inhibited cell differentiation, specific markers for terminal, transit amplifying (TA), and stem cells (ISC) of the intestinal epithelium were analyzed by qPCR. There was a 39% decrease in transcript levels for the ISC marker $Lgr5^+$, and a 49% and 42% decrease in transcript levels in the secretory fated Paneth and Goblet cells, respectively. No significant changes *in trans*cript levels were observed in the absorptive cells, though there was a 30% decrease in absorptive TA cell marker Hes1. Math1 transcript levels expressed by secretory TA cells were also reduced by 31%. Reductions in Lgr5 protein expression via immunohistochemistry results were concordant with qPCR data, and alternations in crypt morphology were also seen in the arsenic exposed animals. To investigate a possible mechanism behind the reduction of intestinal cell types, Indian hedgehog (Ihh) transcript expression was analyzed by qPCR. Ihh is a signal that is expressed in terminal epithelial cells that stimulates the mesenchymal tissue beneath the epithelium to release proliferation and differentiation signals to the ISC and TA cells. Its expression was significantly reduced by 34% in the arsenic exposed animals. To further investigate the significance of Ihh pathway impairment, target genes responsible in cell proliferation and differentiation, Gli1 and BMP4 respectively, will be analyzed by

qPCR and immunoblotting. The results show that intestinal stem cell differentiation is negatively impacted by arsenic in drinking water, potentially due to disruption of the Ihh.

2627 Effects of Arsenic (As)-Contaminated Soil Exposure on Gut Bacteria and Its Relationship with Oral Bioaccessibility of As Using a Multi-Compartment *In Vitro* Gastrointestinal Model

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The World Health Organization and the Agency for Toxic Substances and Disease Registry describes As as a "significant potential threat to human health" due to high toxicity and worldwide prevalence that results in exposure of millions of people. Understanding factors that affect As bioaccessibility and the effects of ingested As on the gastrointestinal system provides insights into risk from exposure and the potential for adverse health effects. We developed a novel multi-compartment (stomach, small intestine, and colon) simulated gastrointestinal model system (GIMS) that is inoculated with microorganisms sourced from ceca or feces of mice. During four weeks post inoculation, we used 16s rRNA gene sequencing to characterize bacterial composition of the colonic phase before and after aliquots of fluids from the GIMS were exposed to 50 ppm of As in contaminated soils. We also determined percent bioaccessibility of total As at each phase of digestion (i.e. stomach, small intestine, and colon) using instrumental neutron activation analysis. After inoculation with fecal bacteria (GIMS fecal) or cecal bacteria (GIMS cecal), bacterial composition of the colon stabilized at weeks 3-4. After stabilization, GIMS fecal colon fluid contained six different bacteria families from four phyla before and after exposure to arsenic-contaminated soils. After As exposure, more As dissolved in GIMS fecal stomach phase than in either small intestine or colon phases. After system stabilization, GIMS cecal colon fluid contained nine different bacteria families from four phyla in the no exposure group alone. Family Rikenellacaeae within phylum Bacteroidetes, made up >5% of the total bacteria composition in the no exposure group at weeks 3-4. After exposure to arsenic, family Rikenellaceae, were greatly reduced in one arsenic-exposed group and absent in three other arsenic-exposed groups. As was found to disrupt gut bacteria composition in an *in vitro* system inoculated with cecal bacteria. The effect of As-induced changes in cecal microbiota on As bioaccessibility is under investigation. *This abstract does not reflect US EPA policy.*

2628 Inhibition of Cellular Proliferation Effects the Sub-Type of Urothelial Cancer Caused by Arsenic Exposure

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Environmental exposure to arsenite (As^{+3}) has a strong association with the development of human urothelial cancer (UC). This cancer is the 5th most common cancer in men and the 12th most common cancer in women. Muscle invasive urothelial cancer (MIUC) can be grouped into basal or luminal molecular subtypes based on their gene expression profile. The basal subtype is more aggressive and can be associated with squamous differentiation that is characterized by high expression of several keratins (KRT1, 5, 6, 14, and 16) and epidermal growth factor receptor (EGFR) within the tumors. The luminal subtype is less aggressive and is predominately characterized by elevated gene expression of peroxisome proliferator-activated receptor-gamma (PPARγ), FOXA1, and GATA3. We have previously shown that As^{3+}-transformed urothelial cells (As-T) exhibit a basal subtype of UC expressing genes associated with squamous differentiation. In this study, we were interested in determining if the molecular subtype of the As-T cells could be altered by inducing the expression of PPARγ and inhibiting the proliferation of the cells. For this purpose the As-T cells were treated with Troglitazone (TG, PPARG agonist, 10 μM), PD153035 (an EGFR inhibitor, 1 μM) or a combination of TG and PD for 3 days. The results obtained demonstrate that treatment of the As-T cells with TG upregulated the expression of PPARγ and FOXA1 whereas treatment with PD decreased the expression of some of the basal keratins. However, a combined treatment of TG and PD resulted in a significant decrease in the expression of basal keratins (KRT1, KRT5, KRT6, KRT14 and KRT16). Thus, our data suggests that inhibition of proliferation facilitates the upregulation of genes involved in maintaining the luminal subtype of UC and this may have implications in treatment of UCs. *In vivo* animal studies would be needed to address the efficacy of using proliferation inhibitors and/or PPARγ agonists to reduce tumor grade/stage of MIUC.

2629 Effects of Arsenicals (Asi, MMA, and DMA) on the Cell Cycle in Bladder Cells HTB-2/HAS3MT

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Arsenic (As) is a ubiquitous element, it is found in both organic and inorganic form, the latter being the most toxic. The As is ingested, undergoes a process of biotransformation by the arsenic enzyme 3 methyl transferase (As3MT). The effects of As on health are multiple presenting non-carcinogenic and carcinogenic effects and the toxic effects reported are only for inorganic As (As), so the objective of this study was to evaluate the effect of arsenical metabolites (Thus, MMA and DMA) on the cell cycle, in a bladder cell line (HTB-2 / HAS3MT). To determine the toxic effects of inorganic arsenic and its metabolites, different tests were carried out with two cell lines, of bladder epithelial origin, the first used as a control, HTB-2 / WT and the second which is stably transfected with the gene of the As3MT, named as HTB-2 / HAS3MT. The cell viability was determined, obtaining a concentration-response relationship between the different concentrations of sodium arsenite [0.1, 1. 2.5 and 5 μM] and the percentage of living cells at times of 4 and 24 hours. Subsequently, the percentage of cells in the different phases of the cell cycle was determined, performing a concentration response curve, where an increase in the percentage of cells in the S phase was observed, but also an increase in the cells in the G2 / M phase at high concentrations. The generation of reactive species was quantitatively determined, and the results showed a concentration-response relationship when the cells with sodium arsenite were exposed to different concentrations [0, 0.1, 1. 2.5 and 5 μM] at 4 and 24 hours. We can conclude that the toxic effects HTB-2 / HAS3MT are due to the presence of metabolites (MMA and DMA), which have a toxicity greater than Asi.

2630 Arsenic Alters ECM to Impair Skeletal Muscle Regeneration

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Skeletal muscle extracellular matrix (ECM) drives conversion of muscle stem cells into either functional or dysfunctional states. Environmental exposure to arsenic weakens skeletal muscle regenerative capacity, and we tested the hypothesis that arsenic diminishes ECM integrity by altering collagen composition and crosslinking. To identify arsenic-induced ECM changes *in vivo*, mice were exposed to 100ppb arsenic in drinking water for 5 weeks. The tibialis anterior muscles were then injured with cardiotoxin and allowed to heal for 10 days. Analysis of muscle sections revealed that arsenic-exposed tissue repaired poorly with reduced levels of collagen 3 (Col3) and Col6 at the injury site. Treated muscle fibers had smaller cross-sectional area and fewer nuclei with lysyl oxidase (LOX), which contributes to collagen crosslinking, nuclear size and chromatin compaction. These changes were recapitulated in primary myofibroblasts cultured with 20nM arsenic for 24h. The arsenic was removed, and cells were allowed to elaborate ECM for 3d. Treated cells had enlarged nuclei and elevated levels of Col4, while Col3 and Col6 were reduced. Arsenic treatment impairs mitochondrial function to epigenetically alter progenitor cell fate, and we tested whether treatment with SS-31, a mitochondrial protectant peptide, could reverse arsenic effects on the ECM. Myofibroblasts were again exposed to arsenic for 24h, and some cell groups were treated with SS-31 as ECM elaborated. SS-31 dramatically increased arsenic-inhibited Col3 levels. These findings suggest that arsenic may impair muscle regenerative capacity by promoting ECM remodeling and altering LOX and collagen levels. Loss of nuclear LOX is consistent with larger, more open nuclei. SS-31 treatment restored the ECM collagen profile, implicating mitochondria as the target of arsenic-promoted dysfunctional ECM and muscle regeneration. *Supported by NIH grant 5RO1ESO23696-04.*

2631 Arsenic-Perturbed Gut Microbiota Activated Intestinal FXR Signaling and Increased Serum Ceramide Levels in Mice to Cause Glucose Intolerance

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The gut microbiota plays a key role in arsenic toxicity, as we and others have demonstrated in a number of recent studies. In particular, arsenic exposure disturbs gut microbiota community composition and alters functional genes and metabolites. Meanwhile, arsenic is known to induce glucose intolerance. However, it is still unclear whether and how the arsenic-disturbed gut microbiota causatively affects glucose homeostasis. To address this gap, we transplanted the gut microbiota from mice with arsenic-induced glucose intoler-

ance to microbiota-depleted mice, followed by glucose tolerance tests. Mice received arsenic-perturbed gut microbiota had impaired glucose tolerance, similar to what observed in donor animals exposed to arsenic. This finding clearly shows that the arsenic-perturbed gut microbiota causatively led to glucose intolerance. In addition, the gene expression level of *Fgf15* and *Shp* in distal ilea was significantly higher in mice with arsenic-perturbed gut microbiota than mice transplanted with normal gut microbiota, which indicated the activated intestinal FXR signaling. Moreover, the gene expression level of multiple ceramide synthases, including *CerS4*, *CerS5*, and *CerS6*, was significantly increased in the distal ilea of mice transferred with arsenic-perturbed gut microbiota, consistent with increased ceramides in sera. Ceramide synthases are the downstream genes of FXR signaling in intestines. In adipose tissues of mice transplanted with arsenic-perturbed gut microbiome, we found that the expression of key genes that regulate glucose homeostasis was significantly changed, including the down-regulated *Chrebp* and up-regulated *Pck2*, which could promote the development of glucose intolerance. Taken together, our current study demonstrates that the causative role of gut microbiota in arsenic-induced glucose intolerance for the first time, and provides a potential molecular mechanism about how arsenic-perturbed gut microbiota may affect host glucose homeostasis.

2632 Arsenite Alters Structure and Function of Alternative Splicing Regulator ZRANB2 by Displacing Zinc from Zinc Finger Motifs

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Exposure to arsenic, a class I carcinogen, affects 200 million people globally. Skin is the major target organ but molecular etiology remains unclear. Arsenite (As^{3+}) can displace zinc (Zn^{2+}) from C3H1 and C4 zinc finger domains, affecting protein function. Zinc finger proteins play key roles in RNA binding and splicing. Using an established HaCaT cell line model of arsenic-induced squamous cell carcinoma, we demonstrated occurrence of global dysregulation of alternative splicing during carcinogenesis. ZRANB2, an alternative splicing regulator with two C4 zinc knuckle motifs integral to its structure and splicing function was chosen as a candidate for this study. We hypothesized that As^{3+} could potentially displace Zn^{2+} from ZRANB2 possibly altering its structure, expression and splicing function. As^{3+}/Zn^{2+} binding (One-site specific binding non-linear model) and mutual displacement experiments (log(inhibitor) vs. response non-linear model) were performed with synthetic apo-peptides corresponding to each ZRANB2 zinc finger motif. A combination of intrinsic fluorescence, UV spectrophotometry, zinc colorimetric assay and ESI-MS/MS was used to characterize metal binding/mutual displacement. ZRANB2 expression in HaCaT cells acutely exposed to As^{3+} (0 or 5 μM; 0-72 h) was examined by RT-qPCR and immunoblotting. TRA2B splicing was monitored by RT-PCR. As^{3+} was found to bind to, as well as displace Zn^{2+} from, each zinc finger motif ($p<0.05$; ANOVA). Also, Zn^{2+} displaced As^{3+} from As^{3+}-bound zinc finger motif acutely ($p<0.05$; ANOVA) but long incubation favored As^{3+} binding. As^{3+} exposure induced ZRANB2 protein expression between 3-24 h ($p<0.05$; ANOVA), but not steady state ZRANB2 mRNA. ZRANB2-directed TRA2B splicing was impaired ($p<0.05$; ANOVA) between 3-24 h post exposure. Arsenic exposure displaces Zn^{2+} from ZRANB2 zinc fingers, changing its structure and compromising splicing of its targets. ZRANB2 protein expression increases in response. Arsenic exposure could be modulating global alternative splicing profile by dysregulating ZRANB2 and other splice regulators, many of which are zinc finger proteins.

2633 Assessing the Role of Chronic Arsenic in Disrupting the EGFR-Signaling Axis

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Lung cancer is the leading cancer in the US, and Kentucky leads the nation with the highest lung cancer death rates. Although smoking is associated with lung cancer development, 20% of patients who die from lung cancer in the US, have never smoked, suggesting there are other risk factors. One of these risk factors is arsenic exposure. Fifteen percent of the US population drinks domestic well water, and in many areas, this unregulated well water contains levels of arsenic that exceeds the Environmental Protection Agencies (EPA) recommended levels. *Though there is a strong association between arsenic exposure and lung cancer development, the clear mechanism is unknown.* Further, previous studies have shown acute arsenic exposure increases expression of the epidermal growth factor receptor (EGFR), a cell surface receptor tyrosine kinase that is associated with many different types of cancer, including lung cancer. We hypothesize that chronic arsenic exposure disrupts the EGFR endocytic trafficking, leading to increased receptor expression. This project examines the impact of chronic "environmentally relevant" levels of arsenic on the EGFR expression, distribution and trafficking. A non-malignant human bronchial epithelial cell line, Beas-2B cells were exposed to 100 nM sodium arsenite for 24 weeks. This concentration was chosen, as it is the average blood arsenic level in individuals who are exposed to high levels of arsenic. The chronic arsenic exposure increased EGFR protein expression levels and its activity, increased transcription and protein levels of TGFα, and altered the distribution of the EGFR. TGFα, unlike the other EGFR ligands, diverts the stimulated receptor from lysosomal degradation. Consistent with an increased protein level of TGFα, we observed increased cell surface level of EGFR in response to chronic arsenic exposure.

2634 Increased Protein O-GlcNAcylation Is a Driver of Arsenic-Promoted Diabetes

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Inorganic arsenic (iAs) is a metalloid found ubiquitously throughout the environment. Chronic exposure to unsafe levels of iAs, greater than the World Health Organization recommended limit of 10 μg/L, is linked to an increased risk of numerous diseases, including type II diabetes. Exposure to pathogenic levels of iAs typically occurs through ingestion of contaminated food or drinking water, and affects populations globally, including the United States, Mexico, Chile, Taiwan, India, and Bangladesh, among others. A key component of metabolic diseases such as type II diabetes, is a shift from normal homeostatic metabolism to a new metabolic program that is pro-pathogenic. As such, generating a better understanding of the metabolic changes that occur during the progression of these diseases, particularly upon exposure to environmental toxins, is vital to enhancing current and future patient therapies. One key metabolic pathway linked to the progression of metabolic diseases is the hexosamine biosynthesis pathway (HBP), which utilizes ~2-5% of the cell's glucose to produce UDP-GlcNAc, a key metabolite used in the O-GlcNAcylation of target proteins. Importantly, altered HBP activity and protein O-GlcNAcylation have both been shown to contribute to the pathogenesis of type II diabetes. Here, we show that chronic exposure to iAs (up to 20 weeks) enhances global protein O-GlcNAcylation in differentiated 3T3-L1 adipocytes and C2C12 myoblasts. Mechanistically, iAs increases UDP-GlcNAc levels, presumably via increased expression of Glutamine-Fructose-6-Phosphate Transaminase 1 (GFPT1), the rate limiting enzyme of the HBP. Further investigation of O-GlcNAcylated targets revealed proteins involved in glucose metabolism, mitochondrial function, vesicle trafficking, and proteostasis, among others. Accordingly, knockdown of OGT, the enzyme responsible for the addition of UDP-GlcNAc to target proteins, partially ameliorated iAs-induced deficits in Glut4 translocation, glucose uptake, and insulin signaling, inferring that iAs-induced changes in HBP activity and protein O-GlcNAcylation could be key mediators of iAs-promoted diabetes. Due to the well-established link between iAs exposure and an increased prevalence of diabetes, clarifying the effects of iAs on the pathophysiological alterations that contribute to key diabetic phenotypes will prove extremely valuable in the generation of novel therapeutic strategies for exposed populations.

2635 Participation of the Nrf2-Keap1 Pathway in the Protective Effect of Curcumin in a Lymphoblast Cell Line (NL-49) Exposed to Inorganic Arsenic

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Inorganic arsenic (iAs) is a metalloid that is found naturally in water tables by polluting drinking water. iAs causes various adverse effects both carcinogenic and non-carcinogenic, mainly through the generation of reactive oxygen species (ROS). The transcription factor Nrf 2 is a master regulator in response to oxidative stress, by promoting the expression of antioxidant and detoxifying enzymes. The objective of this work was to evaluate the protective role of curcumin in lymphoblasts (NL-49) exposed to iAs through the Nrf2 -Keap1 pathway Lymphoblasts were treated with curcumin (5, 10 and 15 μM) at different times (12, 18 and 24 hours) and subsequently with sodium arsenite (5,10 and 20 μM) for 15 hours, assessing cell viability, Nrf-2 target genes and cell cycle. The results obtained allowed us to identify that the concentration of 5 μM curcumin was the least toxic and 10 μM arsenic as the concentration capable of inducing cell death below of 50%. Treatment with 5μM of curcumin for 9 hours, prior to exposure with 10 μM of arsenic, showed a loss of cell viability induced by arsenic, as well as cytostatic effects in the G1 phase of the cell cycle. In addition, it was observed that most of the genes analyzed are expressed after 9 hours of treatment and begin to decrease at 18 hours. Then it can be concluded that curcumin sensitizes the cell by increasing the damage generated by arsenic, decreasing cell viability and increasing apoptosis, despite inducing the expression of Nrf2 regulated genes.

2636 Using the Zebrafish Model System to Determine the Toxicity Interaction of Arsenic and Lead

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Metals such as arsenic (As) and lead (Pb) are environmental pollutants, often found in common sites due to their ubiquitous nature and production of virgin Pb. Both As and Pb are heavy metals linked to adverse health effects; more specifically, As and Pb lead to cardiovascular toxicity and neurotoxicity, among various other effects including cancer and nephrotoxicity. A variety of studies have determined the individual toxicity, developmental toxicity, and biomarkers for the detection of toxicity induced by low dose exposures. Unfortunately, a majority of these studies have been on a single component or in mixture compositions that exceed a binary mixture of just Pb and As. This specific mixture of As and Pb may produce additive / synergistic effects due to their common pathways for neurotoxicity. This study aims to evaluate the interaction between As and Pb to determine what type of toxicity interaction may be present at such concentrations. The concentration addition (CA) model was applied to test the hypothesis that the type of toxicity interaction between Pb and As is additive, due to shared neurotoxicity and vascular toxicity pathways of Pb and As. To determine the toxicity interaction of As and Pb, the zebrafish (*Danio rerio*) biological model was used. The individual chemical studies and the mixture experiments began at 1 hour post fertilization (hpf). Every 24 hrs, survival was recorded. The treatments used for As were 0, 0.1, 0.5, 1, 5, and 10 mM and the treatments for Pb were 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.34, 0.39, 0.43, and 0.48 mM. The LC_{50} values for As and Pb at 120 hpf were 1.74 mM and 0.43mM, respectively. The LC_{50} from these two studies at 120 hpf was used to determine the mixture concentrations. The mixture assessment involved three separate exposure paradigms: experiment 1: [As LC_{25} + Pb LC_{75}, As LC_{50} + Pb LC_{75}, As LC_{75} + Pb LC_{75}, Pb LC_{75}, As LC_{25}, As LC_{50}, As LC_{75}]; experiment 2: [As LC_{25} + Pb LC_{50} , As LC_{50} + Pb LC_{50}, As LC_{75} + Pb LC_{50}, Pb LC_{50}, As LC_{25}, AsLC_{50}, As LC_{75}]; and experiment 3: [As LC_{25} + Pb LC_{25} , As LC_{50} + Pb LC_{25}, As LC_{75} + Pb LC_{25}, Pb LC_{25}, As LC_{25}, As LC_{50}, As LC_{75}]. The pH for all treatments was adjusted to 6.5 in order to avoid precipitation of Pb in solution and to maintain an optimal pH for zebrafish development. The mixture experiments indicate an additive toxicity interaction for As and Pb. Overall, this study serves as a foundation for future studies at lower mixture concentrations to determine if additive toxicity is also observed for specific neurotoxicity and vascular endpoints.

2637 Exposures to Trivalent Arsenicals Alter Metabolomics Profiles in β-Cells and Isolated Pancreatic Islets

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Inorganic arsenic (iAs) is a diabetogen, but mechanisms underlying its diabetogenic effects are poorly understood. Exposure to arsenite (iAs^{III}) and its metabolites, methylarsonite (MAs^{III}) and dimethylarsinite ($DMAs^{III}$), have been shown to inhibit glucose stimulated insulin secretion (GSIS) in β-cells and isolated pancreatic islets. GSIS is regulated by complex mechanisms that depend on metabolism of glucose and other energy producing pathways. The present study used UHPLC High Resolution Orbitrap MS Metabolomics to characterize metabolic fingerprints of β-cells (INS-1 832/13 insulinoma cells) treated with 2 μM iAs^{III}, 0.2 μM MAs^{III} and 2 μM $DMAs^{III}$, and murine islets treated with 2 μM iAs^{III}, 0.25 μM MAs^{III} and 0.5 μM $DMAs^{III}$. Treatment with iAs^{III} and $DMAs^{III}$ degreased GSIS in β-cells, but only the effect of $DMAs^{III}$ was statistically significant. GSIS in islets was significantly inhibited by all three arsenicals. Supervised orthogonal partial least squares discriminant analysis revealed that $DMAs^{III}$ treatment resulted in major metabolic perturbations in β-cells affecting 37 metabolites, as compared to 9 and 5 metabolites perturbed by iAs^{III} and MAs^{III}, respectively. Two metabolites, acetylcarnitine and succinic acid, were decreased following exposure to each of the three arsenicals. Pathway analysis revealed 25 metabolic pathways enriched by treatment with $DMAs^{III}$, including multiple pathways of amino acid transport and metabolism. Six and 17 pathways were enriched by exposure to iAs^{III} and MAs^{III}, respectively. The D-glucuronic acid pathway was the only pathway altered by all three arsenicals. In spite of their significant effects on GSIS, the overall impact of arsenical exposures on the metabolome of islets was less than that in β-cells. Several metabolites were altered by exposure to one or more arsenicals in both β-cells and islets, including acetylcarnitine, glutamate, suberic acid, glutathione and ornithine. The pathways of carbohydrate and amino acid metabolism were among the pathways most affected in both β-cells and the islets. Results of this study will inform future research of mechanisms by which iAs exposure impairs GSIS in β-cells.

2638 Arsenic (3) Methyltransferase (As3MT) Auto-Methylation Primes Arsenic Methyl-Transferase Activity?

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Arsenic toxicity is a global concern to human health and is related to increased incidence of cancer, bronchopulmonary and cardiovascular diseases in exposed populations. In human and mouse, inorganic arsenic (iAs) is metabolized in a series of methylation steps catalyzed by arsenic (3) methyltransferase (As3MT), forming methylarsenite [MAs(III)], dimethylarsenite [DMAs(III)] and the volatile trimethylarsine [TMAs(III)]. Methylated intermediates may be more toxic than inorganic arsenite, and we have previously shown that As3MT is required for arsenic-enhanced atherosclerosis in mice. The methylation of arsenic is coordinated by three conserved cysteine residues proposed to participate in catalysis, namely Cys62, Cys157, and Cys207 in mouse As3MT. The current model of iAs methylation requires a series of intramolecular disulfide bonds formed before the enzymatic methylation of arsenite (iAs3+). In presence of GSH, these disulfide bonds are reduced, leading to the methylation of the iAs in presence of the cofactor S-adenosyl methionine (SAM). Using *in vitro* methylation assays in the absence of arsenic, we find that As3MT undergoes an initial automethylation step before iAs binding. This methylation is enhanced by GSH or DTT, suggesting that reducing cysteine bonds prepares As3MT for automethylation. Following the addition of iAs, the automethylated As3MT is decreased, as transfer of this methyl group completes the first round iAs methylation. Furthermore, using a Flag-As3MT immunoprecipitation coupled to MS/MS, we identify both Cys33 and Cys62 as an acceptor of the methyl group *in vivo*. Site-directed mutagenesis (Cys to Ala) reveals that three of the previously described cysteines are required for the automethylation step. Altogether, our results suggest a new mechanism of arsenic methylation that requires an additional step of As3MT automethylation. Additional *in vitro* and *in vivo* biochemical analyses will be necessary to define the role of the As3MT automethylation in arsenic species formation and/or putative target of As3MT.

2639 Cardiac-Specific SERCA Knockout Mice Replicate Adverse Cardiac Effects of Bromine and Demonstrate Enhanced Myocardial Injury

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Toxic halogens such as bromine are finding increased industrial applications and their storage and transport in large quantities poses risk of accidental or deliberate spills. Our studies have demonstrated that acute inhalation of high concentrations of bromine causes death by cardiopulmonary failure. The toxicity of halogens is mediated by halogenation and inactivation of important cardiac calcium pumps such as the sarcoendoplasmic calcium ATPase, SERCA2. SERCA inactivation causes increased calcium overload and activation of cytosolic calcium specific proteases such as calpains that damage the myocardium. The myocardial damage causes biventricular dysfunction and hypertrophy. To delineate mechanisms, we utilized cardiac specific SERCA knockout mice and exposed them to bromine. Decreased cardiac SERCA itself manifested in increasing the cardiac injury markers, such as troponin I and cardiac calpains. Cardiac specific knockout also demonstrated abnormal contractility. Moreover, exposure of these mice enhanced toxicity to bromine and caused increased damage to cardiac tissue leading to cardiac dysfunction and death. Further impairment in myocardial relaxation in SERCA knockout mice was evident after bromine exposure as demonstrated by hemodynamic analysis. Since loss of cardiac SERCA function and calcium overload have been implicated in the pathogenesis of several cardiac diseases, these studies predict susceptibility of individuals with preexisting cardiac disease to bromine/halogens.

2640 *In Vitro* Prediction of Cardiotoxicity by Simultaneous Evaluation of Multiple Endpoints

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have shown promise for evaluating the electrophysiological effects of drugs. While perturbation of the heart's electrophysiological rhythm is a common

mechanism of drug-induced cardiotoxicity, many cardiotoxic compounds act through other mechanisms that might not be detected by solely measuring electrophysiological endpoints. Analysis of electrophysiology with microelectrode array (MEA) is a nondestructive, label-free technique that permits the evaluation of additional endpoints in the same experiment. We evaluated the effects of test compounds on cellular electrophysiology (MEA), viability (lactate dehydrogenase [LDH] release), and secretion of cardiac damage or inflammation biomarkers (MILLIPLEX® magnetic beads). Test compounds included well-characterized cardiac ion channel modulators (e.g., E-4031 and nifedipine) and mitochondrial toxicants (i.e., potassium cyanide [KCN], phosphine [PH_3], and hydrogen sulfide [H_2S]). Doxorubicin was included as a positive cytotoxic control. Exposure to H_2S or PH_3 was achieved by adding sodium hydrosulfide (NaHS) or zinc phosphide (Zn_3P_2) to the cell culture medium. MEA recordings were taken before and after compound addition; each ion channel modulator induced effects on electrophysiological parameters (i.e. beat period, beat amplitude, and field potential duration) that were consistent with previously reported results. Cyanide induced a robust decrease in beat period and beat amplitude. KCN, Zn_3P_2, and NaHS each triggered elevated levels of IL-8 at 24 hr, with a subsequent reduction at 48 hr. Robust increases in levels of some secreted biomarkers of cardiotoxicity (i.e., CKMB, FABP3, and troponin I) were observed at 24 hr and 48 hr with the highest concentrations of KCN, Zn_3P_2, and NaHS. With the exception of elevated levels of troponin I induced by E-4031 and nifedipine, no robust changes in biomarkers were observed in the cells treated with ion channel modulators. The highest concentrations tested of doxorubicin, KCN, Zn_3P_2, and NaHS each induced significant cytotoxicity that increased from 24 hr to 48 hr for all except Zn_3P_2. Collectively, these results demonstrate the utility of multiplex cardiotoxicity assays that can detect diverse mechanisms of toxicity. Further study is warranted to optimize the assay conditions and endpoints for optimal prediction of chemical-induced cardiotoxicity.

2641 Enhancing Aged Acetylcholinesterase Turnover by Targeting Members of the Enzyme Complex

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Organophosphate (OP) compounds are used as chemical weapons and pesticides. OP species are very potent inhibitors of cholinesterases (ChE), wherein OPs phosphylate the primary active site serine. The phosphate moieties from agricultural chemicals, like parathion, and nerve agents, such as sarin, become fixed in acetylcholinesterases (AChE) in minutes preventing reactivation by available oxime countermeasures. These "aged cholinesterases" contribute to glutaminergic hyperstimulation resulting in muscle contractions, respiratory depression, seizures, and possibly death. Current drug development strategies include generating compounds that can realkylate the phosphate moiety on ChE followed by administration of oximes or introducing increased ChE to combat the elevated levels of acetylcholine. However, no studies to date have tried to exploit endogenous cellular mechanisms to eliminate the impaired ChE. We have developed an on-cell western (OCW) for AChE that permits relative quantitation of protein levels on the surface of cells expressing AChE. When paired with an AChE biochemical assays, we can determine the relative amounts of the active enzyme on the cell surface. Using C2C12 mouse myoblast-like cells, we used the AChE OCW to screen chemicals that could remove diisopropylphosphofluoridate (DFP) aged AChE from the surface of cells. Candidate compounds for further study would be those that reduced aged AChE levels without impacting cellular viability and, after washing the compound from cells, would permit the emergence of active AChE from within cells. Our screen revealed that compounds targeting members of the AChE protein complex were successful at eliminating aged AChE. Furthermore, targeting members of the AChE complex with transient selective protein degradation approaches were also useful in removing aged enzyme. Our presentation will describe some of the targets, evidence, and potential complications of targeting the AChE complex. Nonetheless, our results demonstrate that exploiting the protein biochemistry of AChE and taking advantage of endogenous cellular proteostatic machinery could be a useful approach to solve the problem of aged cholinesterases.

2642 Effects of Hydrogen Sulfide on Brain-Lung-Heart Axis

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Hydrogen sulfide (H_2S) is a toxic gas with a rotten egg smell. Acute exposure to high concentration of H_2S can lead to severe injuries including neurological disorders and acute death. The exact mechanisms underlying H_2S-induced acute death have not been clearly elucidated. Hydrogen sulfide targets multiple organs including brain, lung, and heart. However, there is still a debate about the most critical organ responsible for acute death. In this study the effect of H_2S on the brain-lung-heart axis was evaluated. C57BL/6J mice exposed to 1000 ppm of H_2S by whole body inhalation developed clinical signs such as dyspnea, ataxia, and seizures. Pulmonary edema developed during H_2S exposure and was evident up to 24 h post-exposure. Serum cardiac troponin-I was elevated at 2 h and 24 h post-H_2S exposure, indicating cardiac injury, which was also observed on H&E stained sections. Plethysmography, electroencephalogram (EEG) and electrocardiography were simultaneously collected during H_2S exposure to identify the sequence of the pathophysiology to identify the critical organ responsible to acute death. Respiratory distress and apnea were observed during exposure to H_2S. Respiratory rate and minute ventilation decreased instantly, whereas the tidal volume decreased overtime. EEG results showed that brain activity was significantly and gradually suppressed over time during the H_2S exposure. During seizure episodes, H_2S exposed mice exhibited altered breathing pattern and arrhythmias. Interestingly, during knockdown mice exhibited significantly reduced EEG activity and apnea. Most significant finding was that the breathing pattern was first to be affected. Then brain activity ceased and closely followed by breathing cessation. Consistently, the heart continued to beat after brain activity and breathing had ceased in that order. Pre-treatment with midazolam prevented seizure and knock-down activity, allowing the H_2S exposed mice to avoid sudden loss of consciousness and apnea. An unexpected finding was that midazolam pretreatment significantly reduced pulmonary edema in H_2S-exposed mice. Taken together, these results indicate that brain death precedes breathing cessation and cardiac failure. Therefore, the brain and lung are the most critical organs responsible H_2S-induced acute death. Midazolam was shown to be beneficial both by reducing pulmonary edema and preventing seizure and knock-down activity. These results strongly suggest that countermeasures to increase survival should be directed at mitigating H_2S-induced neurotoxicity and improving respiratory function.

2643 *In Vitro* Degradation and *In Vivo* Biotransformation of Cyanide Antidote Candidate Dimethyl Trisulfide

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Cyanide (CN) is a potent Cytochrome c Oxidase inhibitor resulting in lactic acidosis. Dimethyl trisulfide (DMTS) is a highlighted sulfur donor type cyanide antidote which can react with CN to form the less toxic thiocyanate. Literature data shows that dialkyl polysulfides can go through disproportionation, reduction, and oxidation reactions *in vitro*. The respective products are dialkyl polysulfides, thiols/hydrogen sulfide, and thiosulfonates/thiosulfinates. From the disproportionation reaction of DMTS, we identified dimethyl disulfide (DMDS) and dimethyl tetrasulfide (DM4S). The product of the oxidation reaction of DMDS was identified as S-methyl methanethiosulfonate. The further disproportionation of DM4S resulted in DMTS and dimethyl pentasulfide (DM5S). For these degradation species to be observed, longer time (months) at temperatures of at least 37°C were required. Products were analyzed by GC-MS-SPME and when the reference molecule was available, HPLC-UV was utilized. *In vivo* behavior of DMTS: It is known, based on our prior studies, that DMTS is concentrated in red blood cells and almost freely crosses the blood brain barrier to reach the brain. Our earlier studies indicated that in blood DMTS oxidizes hemoglobin to methemoglobin. During our blood absorption studies, DMDS was identified as one of the metabolites. Ongoing studies are focusing on identifying other metabolites in blood and other organs (brain, heart, and liver). Future studies will extend to other organs including pancreas, kidneys, and lungs. *This research was supported by the Counter ACT Program, NIH Office of the Director, and the NIAID, NIH/Department of Defense Interagency Agreement [AOD16026–001–00000/A120-B.P2016–01] and by the Robert A. Welch Foundation [X-0011] at the Sam Houston State University.*

2644 Inhibition of the Serine Hydrolase Fatty Acid Amide Hydrolase by a Sarin Surrogate

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Organophosphates, such as nerve agents, inhibit acetylcholinesterase (AChE) and other serine hydrolases. Inhibition of AChE alone may not explain all of the neurotoxicity observed from acute exposure to nerve agents. Preliminary studies identified the serine hydrolase fatty acid amide hydrolase (FAAH) as being a possible target for the sarin surrogate nitrophenyl isopropyl methylphosphonate, NIMP. FAAH inhibition and resultant anandamide accumula-

tion has been implicated in delayed neurotoxicity and neuronal degeneration. Oximes play an integral role in the treatment of acute nerve agent exposure by reactivating inhibited AChE. Our laboratory developed a series of substituted phenoxyalkyl pyridinium oximes (US patent 9,227,937) that have been shown to provide central neuroprotection through reactivation of AChE in the brain and preservation of neuronal and glial structures from damage in a rat model. Reactivation of AChE alone may not be the only mechanism of neuroprotection provided by the novel oximes. Reactivation of other inhibited serine hydrolases, such as FAAH, during acute nerve agent exposure could provide a secondary neuroprotective mechanism provided by these novel oximes. Rat forebrain homogenates showed up to an 85% decrease in FAAH enzymatic activity in the presence of 1.0 μM NIMP, confirming that FAAH is a target of the sarin surrogate. Using rat forebrain microsomal preparations, an *in vitro* study inhibited FAAH with NIMP and assessed reactivation of FAAH by our lead oxime, Oxime 20. Initial results using western blot techniques suggested that Oxime 20 provided some reactivation as indicated by binding of the probe, FP-biotin. FAAH activity was monitored by HPLC-MS using an exogenous substrate anandamide (50 μM), but reactivation by Oxime 20 was not observed, suggesting that if the active site was restored by the oxime, it might not be functional. *Support NIH U01 NS083430.*

2645 Monitoring Persistent Neurological Damage in a Rat Model of Acute OP Intoxication Using Micro-CT Imaging

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Acute intoxication with organophosphate (OPs) pesticides and chemical weapons can trigger seizures that rapidly transition to *status epilepticus*, which is associated with long-term neuropathology and cognitive dysfunction. Medical countermeasures for OP-induced seizures can prevent lethality and attenuate seizures but do not sufficiently protect against long-term neurological consequences. Therefore, there is a need for non-invasive tools to longitudinally monitor the neurological health of OP-exposed individuals. The goal of this study was to evaluate the utility of *in vivo* micro-computed tomography (CT) imaging for monitoring brain injury in a rat model of acute intoxication with the OP threat agent diisopropylfluorophosphate (DFP). Adult male Sprague-Dawley rats were administered DFP (4 mg/kg, s.c.) followed 1 min later by treatment with atropine sulfate (2 mg/kg, i.m.) and pralidoxime (25 mg/kg, i.m.) to increase survival. At 40 min post-DFP, animals were injected with either diazepam (DZP) or midazolam (MDZ) to attenuate seizure activity. Micro-CT scans were obtained from all subjects at 3 and 6 mo post-intoxication to evaluate mineralization in the brain, a pathology associated with persistent neurodegeneration and cognitive deficits. A subset of DFP animals displayed resistance to seizure activity. This population, referred to as DFP low responders, was not treated with benzodiazepines, but were scanned using micro-CT at 2 mo post-intoxication. In animals that experienced DFP-induced seizures, micro-CT detected significant mineralization in the hippocampus and thalamus of animals at both time points, and indicated that neither DZP nor MDZ protected against this neuropathologic outcome. Interestingly, DFP low responders also presented with significant levels of mineralization in the brain. These findings highlight the feasibility of using micro-CT imaging to detect persistent neurological damage following acute OP intoxication, and suggest that current medical countermeasures do not protect against mineralization in the brain. They also suggest that mineralization in the brain caused by acute OP intoxication is mediated by seizure-independent mechanisms. *This work was supported by the NIH CounterACT program (U54 NS079202) and a NINDS National Research Service Award Fellowship to EAG (F31 NS110522).*

2646 The Role of Nicotinic Receptors in Seizure Activity and Death Triggered by Acute Organophosphate Intoxication in a Mouse Model

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Organophosphate (OP) nerve agents and pesticides can cause *status epilepticus* (SE) and death by inhibiting acetylcholinesterase (AChE). Excessive accumulation of acetylcholine (ACh) at central and peripheral synapses induces a cholinergic crisis as a consequence of overstimulation of nicotinic and muscarinic receptors. The current standard of care for OP intoxication includes atropine to block muscarinic receptors, pralidoxime to regenerate active AChE, and benzodiazepine to terminate seizures. While this medical countermeasure can prevent mortality, survivors often exhibit persistent neuropathology and electrographic abnormalities. It has been posited that the limited efficacy in preventing long-term effects is due to the fact that the current therapeutic approaches do not target nicotinic receptors. To test this hypothesis, we assessed the efficacy of the non-selective nicotinic receptor antagonist, mecamylamine (MEC), as a potential anticonvulsant in a mouse model of acute intoxication with diisopropylfluorophosphate (DFP). Adult male C56BL6/J mice were pre-treated with MEC (0.5-9.5 mg/kg, s.c.) or an equal volume (80-100 μl) of saline vehicle (VEH, s.c.) 10 min prior to administration of DFP (12.7 mg/kg, s.c.). Their seizure behavior was monitored for 4 h and seizure severity was scored using a modified Racine scale. MEC pretreatment dose-dependently reduced or prevented DFP-induced seizure behavior relative to vehicle controls. Post-exposure administration of MEC (9.5 mg/kg, s.c.) 10 min after DFP injection also significantly reduced DFP-induced seizure behavior within minutes. These findings suggest a necessary role for nicotinic receptors in DFP-induced seizure activity. These observations identify nicotinic receptors as potential therapeutic targets, and support further investigation to identify which receptor subtypes are important in this phenomenon. *This work was supported by the NINDS CounterACT Program (grant # U54 NS079202), the Department of Veterans Affairs VR&E Program, and a predoctoral fellowship from the UC Davis School of Veterinary Medicine 2019-2020 Graduate Student Support Program (GSSP).*

2647 DFP Influences Spontaneous EPSP in Hippocampus ca1, Revealing an Essential Role of nAChRs in DFP-Triggered Neural Network Hyperexcitability

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Our previous report indicated that cortical neuronal/glial co-cultures isolated from embryonic or PND 0 rats or mice express AChE and manifest strong muscarinic cholinergic neurotransmission (mAChR) that negatively modulate glutamatergic-mediated excitability. However, these cultures lack detectable changes in patterns of synchronous spontaneous Ca^{2+} oscillations and electrical spike activity in the presence of DFP or other surrogate nerve agents at concentrations well beyond those that fully inhibit AChE. We thus developed an *ex vivo* adult rat hippocampal slice preparation to measure patterns of spontaneous electrical spikes (SES) activity and assess their responses to DFP. Our results demonstrate that nAChR neurotransmission is essential for DFP-triggered hyper-excitability secondary to acute inhibition of AChE localized within cholinergic synapses. Our goal is to Identify ionotropic nAChR antagonists and partial agonists with differing spectra of subtype selectivity that show efficacy towards normalizing acute DFP-triggered cellular and network hyper-excitability, and ameliorate subsequent biomarkers of neuropathology shown to with following DFP-triggered status epilepticus. Perfusion of slices with DFP (3μM) in aCSF increased both the frequency and amplitude of SES after a short delay and these changes were resistant to washout up to 4 hours with aCSF alone. Perfusion of the dihydro-β-erythroidine hydrobromide (DHβE, 15μM), a selective α4-nAChR antagonist or methyllycaconitine (MLA, 5μM), a selective α7-nAChR antagonist) largely restored both SES frequency and amplitude of DFP-modified slices to those measured before introduction of DFP, and the effects were readily reversible. These results suggest functional nAChRs synapses are necessary and sufficient to acutely trigger seizure-like electrical hyperactivity within mature brain circuits secondary to AChE irreversible inhibition. Importantly, subunit selective nAChR antagonist of either α4-nAChR or α 7-nAChR may represent a new druggable target for combination therapy with standard of care GABA receptor antagonist midazolam. Moreover, the multi-well electrode-array slice preparation represents a valuable tool that permits detailed quantitative analysis of electrical spike patterns for threat agents with differing mechanisms of action as well *ex vivo* assessment of brain slices isolated from nerve agent exposed animals. *1U54 NS079202-06.*

2648 Positron Emission Tomography (PET) Analysis of Synaptic Density in the Rat Brain Acutely Intoxicated with Diisopropylfluorophosphate (DFP)

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Acute intoxication with organophosphate (OP) cholinesterase inhibitors can trigger life-threatening *status epilepticus* (SE). Current medical countermeasures can prevent death from OP-induced SE, but do not effectively protect against chronic morbidity. The pathogenic mechanisms of these chronic effects remain unknown but are thought to involve altered synaptic connectivity. Testing this hypothesis involves quantifying synaptic density over time. Histologic techniques are considered the gold standard for quantifying synaptic density in brain sections; however, these techniques are time-consuming and do not allow for longitudinal assessments of synaptic density over time in the same animal. *In vivo* PET imaging overcomes such limitations. The molecule [^{18}F]UCB-H specifically binds synaptic vesicle glycoprotein 2A (SV2A), a

transmembrane protein expressed in synaptic vesicles, and has recently been developed as a PET probe for imaging synapses. The goal of this study was to determine whether synaptic density measured by PET correlates with *ex vivo* Golgi staining and immunohistochemical (IHC) synaptic metrics. Specifically, we assessed changes in presynaptic SV2A and postsynaptic drebrin and gephyrin. Adult male Sprague-Dawley rats (200-250 g) were administered a single dose of DFP (4 mg/kg s.c.) or vehicle (saline) followed by atropine sulfate (2 mg/kg i.m.) and 2-pralidoxime (25 mg/kg i.m.) 1 min later. Animals underwent *in vivo* PET and MRI (for anatomic registration) imaging at 10 and 28 days post-intoxication with a subset of animals euthanized after each time point to collect brain tissue. Hemisected brains were processed for Golgi staining and IHC. Our results indicate a significant decrease in [^{18}F]UCB-H uptake in the hippocampus following acute DFP intoxication, which appears dependent on the severity of DFP-induced seizures. Initial histological assessments indicate similar outcomes. Collectively, these observations demonstrate that acute OP intoxication causes a loss of SV2A binding sites consistent with an overall reduction in synapse density within the hippocampus and further suggest SV2A PET as a viable approach for assessing synaptic density in preclinical models. *Funding provided by NIH CounterACT (U54 NS079202).*

2649 Delayed Treatment with Midazolam, Allopregnanolone, and Perampanel Is Superior to Midazolam Alone in Reducing Spontaneous Recurrent Seizures and Neuroinflammation but Does Not Improve Long-Term Behavioral Deficits in the Rat Model of Acute DFP Intoxication

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Organophosphate (OP) nerve agents are potent neurotoxicants. OPs can trigger seizures that rapidly progress to *status epilepticus* (SE). If not terminated within minutes, OP-induced SE leads to extensive neuropathology and neurological deficits. We have previously shown in a rat model of acute intoxication with the OP diisopropylfluorophosphate (DFP), that treatment with the standard-of-care anti-seizure drug midazolam in combination with allopregnanolone (a neurosteroid that acts as a positive allosteric modulator of the $GABA_A$ receptor) and perampanel (a selective non-competitive AMPA receptor antagonist) more effectively terminated acute electrographic seizures than midazolam alone. To determine whether improved seizure control mitigated chronic morbidity, adult male Sprague Dawley rats were dosed with DFP (4 mg/kg sc) immediately followed by atropine sulfate (2 mg/kg im) and 2-PAM (25 mg/kg im). At 40 min post-DFP, rats received midazolam (1.8 mg/kg im), allopregnanolone (6 mg/kg im) and perampanel (2 mg/kg im). Neuropathology was assessed by immunohistochemistry (IHC) at 1, 3, 7 and 28 d post-exposure; behavioral testing was performed at 1 and 2 mo post-exposure. The combined treatment was superior to midazolam alone in reducing spontaneous recurrent seizures (SRS) and neuroinflammation (GFAP and IBA-1 IHC). In contrast, the combined treatment was no better than midazolam in protecting against neurodegeneration (FluoroJade C staining) or in mitigating reactivity (Irwin reactivity test) or cognitive deficits (novel object recognition and contextual fear conditioning). In summary, the combined treatment was effective in improving some but not all long-term outcomes, and suggests that neuroinflammatory responses may contribute to OP-induced SRS, but not cognitive deficits. *Supported by NIH CounterACT Program (NS079202).*

2650 Pharmacological Inhibition of mTOR Activation by Rapamycin Attenuates Neuroinflammation and Spontaneous Recurrent Seizures in a Rat Model of Acute Organophosphate (OP) Intoxication

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Acute intoxication with OP cholinesterase inhibitors can cause seizures that rapidly progress to status epilepticus. If not treated within minutes of onset, this acute response can result in chronic neurological impairment, including spontaneous recurrent seizures (SRS), via unknown mechanisms. Studies from other labs have implicated enhanced mammalian target of rapamycin (mTOR) activity in neuroinflammatory and epileptogenic processes, suggesting the possibility that mTOR signaling is involved in OP-induced SRS. Therefore, we used a rat model of acute intoxication with the OP, diisopropylfluorophosphate (DFP), to establish the temporal profile of mTOR activation in the brain, and to determine whether pharmacological inhibition of mTORC1 with rapamycin (RAPA) attenuated neuroinflammation and SRS. Adult male Sprague-Dawley rats (200-250g) were administered a single dose of DFP (4 mg/kg sc) followed by atropine sulfate (2 mg/kg im) and 2-pralidoxime (25 mg/kg im) 1 min later. At 40 min post DFP, a subset of animals were administered midazolam (MDZ, 1.8mg/kg im), RAPA (6 mg/kg ip), or both MDZ and RAPA. Animals were euthanized at varying times post-exposure and brains were collected for biochemical analysis of mTOR activation (phosphorylation of ribosomal protein S6) and histological analyses of neuroinflammation (IBA1 and CD68). Western blot analysis revealed upregulated mTOR activity within 1 h post-exposure (HPE) that returned to baseline levels by 3 d post-exposure (DPE) in DFP animals not treated with MDZ/RAPA. Administration of MDZ/RAPA significantly inhibited mTOR activity at 24 HPE and 7 DPE. An additional cohort of animals were administered RAPA (6 mg/kg ip) once daily for 7 d following acute DFP intoxication and MDZ treatment. Daily RAPA treatment suppressed microglial activation at 7 DPE, and initial data from animals instrumented for cortical electroencephalographic (EEG) recording suggest that daily RAPA treatment attenuated SRS development in DFP animals. These studies characterize mTOR activation following OP intoxication and identify mTOR as a potential therapeutic target for mitigating OP-induced SRS. *Funding: NIH CounterACT program (NS079202).*

2651 Phosphylated Oximes Transiently Impair Cellular Respiration and Activate Stress Responsive Signaling

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Organophosphorus compounds (OPs) are a diverse class of compounds used as insecticides and chemical weapons. OPs are potent inhibitors of acetylcholinesterase (AChE) that can trigger a cholinergic crisis in humans resulting in muscle contractions, respiratory depression, seizures, and even death. The current countermeasure consists of atropine and oxime, which reactivates AChE by removing an alkylated phosphate group from the active site. Recent studies suggest that potential noncholinergic targets of OPs can lead to the persistence of symptoms and neurological sequelae long after recovery of AChE activity. To better understand pathology following OP exposures, we assessed the collective impact of OP and oxime on the physiology of cells with and without AChE. We subjected human SH-SY5Y neuroblastoma cells ($AChE^+$)cells, mouse C2C12 myoblast cells ($AChE^+$), and human IMR90 lung fibroblasts (AChE-null) to acute OPs exposures followed by AChE reactivation by oximes. $AChE^+$ cells administered increasing concentrations (up to 50μM) of diisopropylfluorophosphate (DFP) followed by stoichiometric equivalent amounts of pralidoxime (2-PAM) resulted in a transient inhibition (20-30%) of respiration. The inhibition of basal respiration was not observed in cells treated with DFP or oxime alone and was absent in AChE-null IMR90 cells. The transient respiratory impairment occurred with other OPs (i.e. paraoxon-methyl) and oxime (i.e. obidoxime) co-treatments. The magnitude of DFP and 2-PAM respiratory inhibition was increased nearly three-fold by adding a membrane permeabilization agent. Because phosphylated oximes are generated during AChE reactivation and are more lipophilic than unmodified oximes, we synthesized a phosphylated oxime (POX) of DFP and 2-PAM and treated AChE+ and AChE-null cells with increasing concentrations of POX. POX induced a transient inhibition of respiration slightly higher (25-35%) than the co-treatment of DFP and 2-PAM in $AChE^+$ cells, and POX treatment triggered a transient inhibition of respiration (18% on average) in AChE-null cells as well. Digitonin gradients and subcellular fractionation revealed that phosphylated forms of 2-PAM and POX species were present within cells, including inside mitochondria. The respiratory impairment coincided with increased MitoSOX-Red fluorescence and increased levels of phospho-JNK and phospho-p38. These results indicate that phoshphylated oximes can enter cells and affect cellular physiology and activate stress responses.

2652 Using a Novel Humanized KIKO Mouse Model to Investigate *In Vivo* Reactivation of Nerve Agent-Inhibited Acetylcholinesterase

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Organophosphorus nerve agents (NA) are potent acetylcholinesterase (AChE) inhibitors that cause numerous toxic effects, including hypersecretion, bronchoconstriction, convulsive seizures, and death. The ability to reactivate NA-inhibited AChE is critical to protect brain function and improve survival. The US military currently employs the oxime reactivator pralidoxime (2-PAM) as a countermeasure for NA poisoning. While 2-PAM restores AChE activity in the periphery, it cannot cross the blood-brain barrier (BBB) to reactivate NA-

inhibited AChE in the CNS. Here we employ a newly established mouse strain (KIKO) that lacks serum carboxylesterase, an endogenous bioscavenger for NA, and expresses human AChE in lieu of mouse AChE. This model can potentially provide results that are more comparable to the human response to NA countermeasures. In this study, we tested oximes obtained from Southwest Research Institute, SWRI-80 or SWRI-144, at 25, 45, or 63 mg/kg, fifteen min following exposure to sarin (GB), cyclosarin (GF), or VX. Brain regions (B) and peripheral tissues (P) were collected 45 min after oxime administration, and AChE activity was determined using an Ellman assay. AChE activity, expressed as a percentage of the saline/saline control activity, was significantly reduced after exposure to GB (4.5% - 6.4% B; 8.3%- 9.9% P), GF (22.8% - 36.9% B; 14.7%- 48.7% P), and VX (6.8% - 20.9% B; 6.3%- 10.9% P). Both SWRI-80 and SWRI-144 significantly reactivated AChE activity in brain and peripheral tissues. In animals exposed to GB, GF, and VX, the highest dose of SWRI-80 restored AChE activity up to 24.8% B and 51.9% P, 92.8% B and 82.2% P, and 60.3% B and 49.9% P, respectively. Similarly, the highest dose of SWRI-144 reactivated AChE activity to 22.9% B and 34.9% P, 50.8% B and 61.8% P, and 44.6% B and 41.2% P in animals intoxicated with GB, GF, and VX, respectively. Overall our results showed that the KIKO mouse model may be used to test AChE reactivation after NA exposure; moreover, both SWRI-80 and SWRI-144 displayed promise as countermeasures for NA, exhibiting the ability to reactivate NA-inhibited AChE in peripheral tissues and the CNS.

2653 A Comparison of *In Vitro* Reactivation Efficacy of Novel Substituted Phenoxyalkyl Pyridinium Oximes for Organophosphate-Inhibited Human and Rat Acetylcholinesterase

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In vitro tests were conducted to compare the efficacy of 4 novel oximes and pralidoxime (2-PAM) for human and rat acetylcholinesterase (AChE). The novel oximes were from a platform of substituted phenoxyalkyl pyridinium oximes (US Patent 9,227,937). The organophosphates (OPs) tested were a sarin surrogate nitrophenyl isopropyl methylphosphonate (NIMP) and the active metabolite of the insecticide parathion, paraoxon (PXN). The source of AChE was erythrocyte "ghost" preparations from both species; these are composed of washed cell membranes and can be used as a model of AChE in the nervous system since the AChE is the same gene product in both tissues. The human blood samples were purchased from a commercial vendor. Rat blood samples were collected from Sprague Dawley rats. Preparation involved centrifugal separation of the erythrocytes, lysis of the cells and three washes to remove hemoglobin. The reactivation potential of the approved antidote 2-PAM and novel oximes 1, 15, 20, and 55 against NIMP and PXN was determined. Concentrations of NIMP (3.16 μM) and PXN (10 μM) were selected to yield about 80% AChE inhibition during a 15-minute incubation period, followed by oxime (100 μM) for a 30-minute incubation of reactivation. AChE activity was assessed by a modified Ellman method, 1 mM acetylthiocholine as a substrate and DTNB as the chromogen, with three replications to calculate the inhibition and reactivation compared to solvent controls. For NIMP, novel oxime reactivation was similar or higher for all 5 oximes for the human (82-92%) than the rat (80-89%). Additionally, reactivation was higher for all novel oximes than for 2-PAM with NIMP in rat and human samples. For PXN also, novel oxime reactivation was similar or higher for all 5 oximes for the human (20-64%) than the rat (13-64%). With PXN, reactivation with 2-PAM was higher than with the novel oximes. These data indicate that the novel oximes show reactivation efficacy toward OP-inhibited AChE from humans at least as good or higher than for rats, and that the efficacy results that are currently being obtained in rat experiments should be representative of reactivation efficacy that would occur in humans. *Supported by NIH U01:NS107127.*

2654 Novel Pyridinium Oximes in Combination with 2-PAM or Another Novel Oxime Potentiate Survival following Organophosphate (OP) Exposure in Rats

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Acetylcholinesterase (AChE) inhibition by OPs, including nerve agents and some insecticides, results in overstimulation of the nervous system and may lead to death. Traditional therapies for OP exposures include atropine, and an oxime to reactivate inhibited AChE, 2-PAM in the US. Although 2-PAM is an effective AChE reactivator and can increase survival following OP intoxication, it has limited ability to cross the blood-brain barrier (BBB) and reactivate inhibited AChE in the central nervous system. Novel pyridinium oximes (US patent: 9,227,937) synthesized to increase lipophilicity and BBB penetration have been shown to reactivate OP inhibited AChE, attenuate seizure-like behavior, decrease neuropathology and increase survival in rats. The three most efficacious of these oximes were tested in binary mixtures with 2-PAM or one of the other novel oximes in rats administered lethal doses of a sarin surrogate or paraoxon. A sarin surrogate, nitrophenyl isopropyl methylphosphonate, NIMP (0.6 mg/kg) or paraoxon (PXN, 0.8 mg/kg) were administered SC in rats followed by atropine IM (0.65 mg/kg) and binary mixtures of 2-PAM and novel oximes IM (146 μmoles/kg each) at the onset of seizure-like behavior. Novel oximes in combination with 2-PAM yielded 53-87% and 67-93% survival, while combinations of novel oximes yielded 47-87% and 47-60% survival for NIMP and PXN, respectively, in male rats. Similarly in female rats matched for estrus cycle, novel oximes in combination with 2-PAM yielded 67-83% and 73-87% survival for NIMP, and PXN, respectively, while combinations of novel oximes yielded 47-67% survival for NIMP and 47-60% for PXN. Combinations of oximes yielded equivalent or greater survival than a single oxime and attenuated seizure-like behavior. In addition, 24-hour survival was determined for mixtures of 2-PAM and novel oximes in guinea pigs (MRIGlobal) challenged with a LD_{85} of sarin. Guinea pigs were monitored and scored for signs of toxicity. Survival for mixtures of novel oximes and 2-PAM ranged from 63-87%. Toxic signs scores were lower for animals receiving mixtures of oximes. Preliminary safety studies were conducted on the novel oximes in binary mixtures with 2-PAM. Male rats were administered oxime mixtures and observed for 14 days. No signs of overt toxicity were observed. Results suggest these oximes have therapeutic potential for OP exposures. *Support: NIHU01:NS107127.*

2655 Analysis of the Inhibitory Potency, Oxime-Mediated Reactivation Profile, and Binding Characteristics of Metabolites of Phorate

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Organophosphates (OPs), used as insecticides and nerve agents, pose a severe threat to military personnel and civilians because of their potency as inhibitors of acetylcholinesterase (AChE). The OP insecticide phorate (rat oral LD50 1.4-3.7 mg/kg) is particularly toxic and could be used as a chemical weapon. Unlike similar OPs, phorate exhibits an unusual delay in the appearance of toxic signs in laboratory rats. Phorate has a more complex metabolism than most OPs: activation to phorate-oxon (PHO), then sulfoxidation to PHO-sulfoxide (PHX) and then to PHO-sulfone (PHS), which are increasingly more potent anticholinesterases. Additionally, PHO exhibits a different oxime-mediated reactivation profile than the well-studied OP paraoxon (PXN) which is also a diethyl phosphate. Two hypotheses, that PHO is bioactivated to its more potent metabolites PHX and PHS in the brain, and that PHO utilizes an unorthodox ethoxy leaving group, could help explain these differences between PHO and PXN. Analysis of OP scavenger carboxylesterase inhibition in rat liver by phorate metabolites indicates poor inhibition efficacy by PHX (IC50: 420 nM) as compared to PHO and PHS (IC50: 20 or 32 nM, respectively); this could support the hypothesis of an alternative leaving group. Computational modeling (docking, molecular dynamics, quantum mechanics/molecular mechanics) is used to further determine the plausibility of the alternative leaving group hypothesis. Finally, oximes, like FDA-approved 2-PAM, can reactivate inhibited AChE, but 2-PAM is unable to penetrate the blood-brain barrier (BBB). MSU's novel substituted phenoxyalkyl pyridinium oximes (US patent 9,277,937) have been shown to reactivate inhibited AChE in the brain in animal tests. *In vitro* results showed levels of novel oxime AChE reactivation in rat brain preparations inhibited by PHO, PHX, or PHS that are comparable to those of 2-PAM. Initial *in vivo* results showed increased 24-hr survival compared to 2-PAM when novel oximes were administered following lethal doses of PHO in rats. MSU's novel oximes may lead to better protection from phorate poisoning. *Support: NIH R21 NS108954.*

2656 Immediate-Early Gene Expression May Help Explain Hippocampal Neuroprotection of a Novel Phenoxyalkyl Pyridinium Oxime Antidote to Organophosphates

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Past assassinations and terrorist attacks demonstrate the need for a more effective antidote against nerve agents and other organophosphates (OP) which cause brain damage through inhibition of acetylcholinesterase (AChE). Our lab has designed novel phenoxyalkyl pyridinium oximes (US patent 9,277,937) that demonstrate the ability to cross the blood-brain barrier and attenuate hippocampal damage in a rat model (Dail *et al.,* 2019 *Tox Sci* 169:465). This project examined whether expression changes in immediate-early genes might help explain this protection. Sprague Dawley-derived

rats were subcutaneously (SC) given either the vehicle (DMSO) or a high sublethal dosage (0.325mg/kg) of the sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP). One hour later peak inhibition (about 80%) of brain AChE and signs of hypercholinergic toxicity, including seizure-like behavior, occur. At this point, rats were intramuscularly (IM) given 146 µmoles/kg of 2-PAM or novel Oxime 20 in Multisol (48.5% H_2O, 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol) or Multisol alone. The hippocampus was dissected, snap frozen in liquid nitrogen, and stored at -80°C. RNA was isolated with the Qiagen RNeasy Plus Mini Kit and examined by duplex qPCR on a Stratagene MX3005 qPCR system using IDT PrimeTime qPCR Assays and Gene Expression Master Mixes. Expression of genes involved in brain repair (*Bdnf*), astrocyte damage (*Gfap*), initiation of neuronal regeneration (*Fos)*, and apoptosis control *(Jdp2, Bcl2l1, Bcl2l11)* were normalized to the housekeeping gene RPLP1, then compared across the treatment groups; NIMP alone, oxime alone, NIMP followed by oxime, and vehicles. *Gfap*, *Jdp2*, and *Bcl2l11* showed no statistically significant changes in any treatment group. *Fos* and *Bdnf* expression were not significantly altered from vehicle levels by NIMP/Oxime 20 but were significantly decreased and increased, respectively, by NIMP/2-PAM treatment. *Bcl2l1* expression was significantly decreased by NIMP. Although this was reversed by both oximes, Oxime 20 stimulated expression more than 2-PAM and returned it to a level closer to that of the vehicle. Since *Bdnf, Fos* and *Bcl2l1* are all involved in aspects of neuronal survival, their Oxime 20 associated expression could partially explain the observed attenuation of NIMP-related hippocampal damage. *Support: NIH U01NS107127.*

PS 2657 Evaluation of Intramuscularly Administered A1 Adenosine (ADO) Receptor Agonists as a Delayed Countermeasure for Organophosphorus Nerve Agent-Induced Status Epilepticus (SE)

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Organophosphorus nerve agents like sarin (GB) and soman (GD) inhibit acetylcholinesterase, resulting in a buildup of acetylcholine that can lead to sustained seizure activity, or status epilepticus (SE). Previous research showed that activation of A1 ADO receptors inhibited neuronal excitability, which could aid in SE termination. (±)5'Chloro-5'-deoxy-ENBA (ENBA) and 2-Chloro-N6-cyclopentyladenosine (CCPA), two A1 ADO receptor agonists, effectively terminated GD-induced SE in rats when administered via intraperitoneal (IP) injection. However, IP injections are not a clinically relevant route of administration, so the current study evaluated the efficacy of these agonists in terminating GB- or GD-induced SE in rats when administered via intramuscular (IM) injection. Telemetrized adult male Sprague Dawley rats were exposed to either GB (150 µg/kg [1.2 x LD_{50}], SC) or GD (90 µg/kg [0.8 x LD_{50}], SC) and were treated with either ENBA or CCPA at 15, 30, or 60 minutes after seizure onset or left untreated. Neuropathology scores were assessed up to 7 days post-exposure (maximum score of 16). With the GB model and 60-minute treatment delay, ENBA-treated rats experienced 78.6% seizure termination (*N=14*) and reduced neuropathology (6.2 ± 1.85, *N=5*). However, CCPA-treated rats experienced 85.7% seizure termination (*N*=14) but only slightly reduced neuropathology (10.9 ± 2.19, *N*=7) compared to untreated animals with no seizure termination (*N=13*) and severe neuropathology (14.25 ± 1.03, *N*=4). With the GD model and 60-minute treatment delay, ENBA-treated rats experienced 92.8% seizure termination (*N*=14), CCPA-treated rats experienced 78.6% seizure termination (*N*=14), and untreated rats experienced 15.4% seizure termination (*N*=13). Neuropathology results for GD-exposed animals are in progress. While ENBA and CCPA both demonstrated a clear ability to terminate SE when administered up to 60 minutes after seizure onset, ENBA provided more neuroprotection, making it a promising candidate as a delayed countermeasure for nerve agent-induced SE. Future studies examining the efficacy of ENBA in conjunction with current medical countermeasures will be conducted.

PS 2658 Glutathione, Cysteine, and N-acetyl-cysteine Conjugates as Biomarkers of Exposure to Sulphur Mustard for a Specific and Noninvasive Analytical Method

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Sulphur mustard (SM) is a vesicant chemical warfare causing severe skin, respiratory tract and eye damage at very low concentration with long-term effects. Despite the Prohibition of Chemical Weapons Convention, exposure to SM remains relevant to armed conflicts, terrorist acts or casualties with old shells. Therefore, sensitive non-invasive analytical assays to detect specific biomarkers in urine or blood are necessary for many reasons: in forensic science as a proof of contamination, for diagnostic and for prognostic. In the present work, we developed an ultra-high-pressure liquid chromatography - tandem mass spectrometry approach (UHPLC-MS/MS) to quantify adducts between CEES, the monofunctional derivative of SM. We also performed biological experiments to validate their use as biomarkers. SM, a strong bifunctional electrophile agent, rapidly reacts on biomolecules such as DNA, proteins, and glutathione. The monofunctional 2-chloroethyl ethyl sulphide (CEES) is used for this study because it is not as highly regulated as sulphur mustard. In DNA, the major adduct is the N7-guanine derivative. It rapidly depurinates and reaches the extracellular medium. Glutathione is a major detoxifying tripeptide in cells and is converted into CEES conjugates, which are further metabolized in cysteine-CEES and N-acetylcysteine-CEES conjugates. The latter metabolites are eliminated in biological fluids. Emphasis was placed in the work on the optimization of the sample preparation based on Solid Phase Extraction. All analyses were carried out by UHPLC-MS/MS in the "Multiple Reaction Monitoring" scan mode. The overall recovery yield of the SPE was between 70 and 90 % while the limit of detection of the UHPLC-MS/MS method was in the femtomolar range. The quantitative aspect was improved by use of isotopic dilution. We are able to quantify the biomarker N7-guanine adduct in the DNA of HaCaT cells (keratinocyte cell line) exposed 30 minutes to CEES. The same molecule was also detected in the culture medium 6h after the treatment, together with the glutathione and the cysteine S-conjugates. We could thus validate that the treated cells excreted these specific biomarkers. Similar experiments were performed in "fresh" human skin explants exposed to CEES with quantification of the biomarkers 1h, 6h and 24h after the treatment. In each case, the amounts of adduct and conjugates were dose-dependent. Interesting temporal effects were observed. Altogether, it can be concluded that the chosen biomarkers are biologically relevant. They will now be quantified in urine or blood and the assay will be extended to sulphur mustard.

PS 2659 Gaming All-Hazards Routing Theory (GARTH) Determines Probabilistic Risk of Toxic Industrial Chemicals and Materials in Urban Environments

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The evolving landscape of chemical hazards demands that both military and civilian personnel navigate their environment with not only an awareness of current threats, but with the ability to forecast new ones. Toxic Industrial Chemicals (TICs) and Toxic Industrial Materials (TIMs) are of increasing risk to soldiers and civilian first responders, as there is growing concern that these may be exploited by enemy combatants as targets for creating weapons of opportunity. For instance, the common TICs ammonia, sulfur dioxide, and phosgene are stored in large quantities at predictable facilities, making them accessible targets for creating a chemical attack or explosion. These stored compounds could also be disrupted by natural disasters, creating situations requiring equally rapid chemical risk assessment. There is a need for tools that compute probabilistic risks and minimize them with respect to personnel readiness and safety due to these unpredictable hazards. We are developing Gaming All-Hazards Routing Theory (GARTH), a software system which quantitatively assesses environmental chemical threats for optimized navigation in complex environments, including areas of disaster relief, national emergency locations, and warzones. Risk analysis of routes is done by integrating exposure concentrations, routes of exposure, weather patterns, and other aspects of key environmental threats into a single quantification that describes the chance of encountering hazardous conditions in the area. In developing GARTH, we will also expand upon current systems to develop the Toxicological Operational Knowledgebase System (TOKS), which will contain relevant biochemical and toxicological data for better characterizing the biological effects of TICs and TIMs specific to urban environments. With an established route forecasting model and chemical database, our next step is to create an interactive graphics user interface, allowing soldiers and first responders to use GARTH for mission planning and disaster recovery purposes. Users will be able to enter real-time exposure information to update risk assessments and determine probability of risk associated with putative chemical exposures. This initial product will serve as a proof of concept that our algorithm effectively evaluates environmental threats for toxicity risk and makes accurate routing decisions based upon these assessments.

2660 Approaches for Probabilistic Health Risk Estimates during Emergency and Battlefield Situations

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Warfighters and first responders find themselves in rapidly deteriorating situations, where they may be exposed to chemical mixtures rarely seen by the general public. Typically these exposures will include toxic industrial chemicals (TICs) and toxic industrial materials (TIMs). In emergency situations, acute exposure is inevitable and toxicity reference values developed for the general public are generally not applicable. In order to perform better mission planning (e.g., ensuring access to the proper medical countermeasures), commanders need to know the probability of exposed warfighters and first responders to develop a critical acute toxicity that impairs mission readiness. To calculate this probabilistic risk, the commander needs to know 1) the probability the possible exposure levels and 2) the probability that any given exposure will yield a hazard. In this presentation I will demonstrate how I use Bayesian statistical approaches and curve-fitting techniques to calculate the probabilistic risk of developing acute toxicity that will impact the ability to complete the mission using data for the gases phosgene and chlorine. Bayesian approaches work by integrating data from multiple studies together into probability distributions. Curve fitting approaches are used to identify mathematical relationships between acute toxicity and exposure parameters such as concentration and time. By combining these approaches, I have calculated the probability of acute toxicity as a function of concentration and time using data from several studies. Using information about actual or predicted chemical exposure levels at the scene of a chemical release, my software estimates the probability that a warfighters or first responders will develop an acute toxicity of interest. *The opinions expressed are those of the author and do not necessarily reflect the views of the US Army.*

2661 Granulocyte Colony-Stimulating Factor (Neupogen; Filgrastim) Accelerates Neutrophil Recovery after Sulfur Mustard Exposure

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While exposure to sulfur mustard (SM) is commonly associated with the production of vesicating dermal, ocular, and respiratory injuries, systemic damage to bone marrow, spleen, and lymphatic tissue also occurs. These effects decrease critical immune cell populations leading to higher susceptibility to infection and life-threatening septicemia. There are currently no approved medical countermeasures for SM-induced myelosuppression. An intravenous (IV) SM challenge model was developed in adult Sprague Dawley rats to evaluate the efficacy of immunostimulants such as Neupogen® (filgrastim) to rescue SM-induced leukopenia. The IV SM challenge model was used to isolate the hematologic toxicity from potentially confounding effects arising from dermal, ocular, or respiratory injuries. Characterization of injury progression through 35 days after IV SM exposure found toxic signs concurrent with body weight loss and marked decreases in complete blood cell counts. Early-onset lymphopenia 1-day post-exposure was followed by delayed neutropenia and thrombocytopenia at 5-7 days post-exposure. Flow cytometry of circulating blood revealed changes in B- and T-cell populations, accompanied by loss of mesenchymal and hematopoietic bone marrow stem cells. Prior to efficacy testing, pharmacokinetic/pharmacodynamic (PK/PD) analyses were performed in naïve rats to select the apparent human equivalent dose of Neupogen®, administered as a single dose or daily for 8 days. The $t_{1/2}$ for Neupogen® was ~3 hr, and drug concentrations did not increase with repeat dosing, while PD analyses showed that 8 daily doses resulted in neutrophil counts as much as 32-fold higher than pre-dose values and 2-4 fold higher than a single dose. When administered at ~24 hours after SM-challenge, daily Neupogen® treatment did not prevent delayed-onset neutropenia. However, Neupogen®-treated animals recovered from SM-induced neutropenia and body weight loss faster than vehicle controls. Collectively, this work 1) Corroborates results of a previous pilot large animal study, 2) Validates the utility of a small rodent IV SM screening model, and 3) Provides compelling evidence for the potential clinical utility of Neupogen® as an adjunct treatment following SM exposure. *Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of Battelle, SRI, NIH, or the US Government.*

2662 Rabbit Conjunctiva as a Target for Ocular Injury Induced by Sulfur Mustard

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Sulfur mustard (SM; bis(2-chloroethyl) sulfide) is a bifunctional alkylating agent that has been used in chemical warfare. SM can induce ocular irritation, tearing, pain, photosensitivity, and short-term blindness depending on the dose and duration of exposure. In the present studies, we examined the effects of neat SM (0.4 microliters applied to the central cornea) on the conjunctiva of New Zealand white male rabbits. Twenty-eight days post-SM exposure rabbits were euthanized, eyelids removed and prepared for histology and immunohistochemistry. Following SM exposure, H&E staining revealed erosions in the epithelial surface of the conjunctiva, squamous metaplasia, engorged lymph nodes and an inflammatory cell infiltrate in the adjacent dermis, while Gomori's trichrome staining showed compacted dermal collagen. In control tissue, proliferating cell nuclear antigen (PCNA), a marker of cellular proliferation, was expressed in conjunctiva cell nuclei surrounding goblet cells; this was continuous throughout the basal layer. In SM-exposed rabbit eyelids PCNA was upregulated in the nuclei of cells in the hyperplastic conjunctiva, in cells surrounding goblet cells, and in cells in the basal epithelium. SM-induced PCNA expression was also expressed in the nuclei of cells in the dermal inflammatory cell infiltrate, and in nuclei of cells in the conjunctival-dermal associated appendages including lymph nodes and accessory lacrimal gland acini. pH2.AX, a marker of DNA double-strand breaks, was also expressed in PCNA expressing lymph nodes of SM-exposed rabbit eyelids. Keratin 17, a marker of epithelial cell wound repair, was constitutively expressed in basal cells of the conjunctiva in control eyelids while it was upregulated throughout the hyperplastic conjunctiva, in the accessory lacrimal glands, at the transition zone of the junction between the conjunctiva, and in the cornified epithelium. These data indicate that SM injury to the conjunctiva may in part modify epithelial cell function leading to impaired ocular barrier integrity. *Supported by NIH grants AR055073 and ES005022.*

2663 Epithelial Repair in the Rabbit Cornea following Exposure to Sulfur Mustard

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Sulfur mustard (SM; bis (2-chloroethyl) sulfide) is a potent vesicating agent that has been used in chemical warfare. Exposure of the eyes to SM can cause corneal erosions, epithelial defects, neovascularization, microblistering, and/or dry eye disease, all injuries that can lead to corneal epithelium sloughing and ultimately blindness. In the present studies, the effects of ocular exposure to SM vapor (0.420 mg/L for 8 min) on the corneas of male New Zealand white rabbits was characterized at 1, 3, 7, 14, 21 and 28 days post-exposure. During this time, corneal thickness increased from 400 microns to 900 microns (14d), then decreased to 600 microns (28d). Unexposed control corneas were clear throughout the time course. However, after SM exposure, increased corneal thickness was associated with increased corneal opacity and neovascularization. Histologically, SM exposure induced epithelial-stromal separation at 3 days, by which time 16% of the epithelia had separated from the stroma, and by 28 days detachment had decreased to 9%. Also observed was post-SM epithelial thinning, increased stromal disorganization, and edema. Sporadic keratocytes were also evident in the edematous stroma by day 14. At 28 days post-SM, an inflammatory cell infiltrate was observed in the stroma. In control corneas, keratin 17, a marker of wound repair, was constitutively expressed in the limbus and bulbar conjunctiva. By 14 days post-SM, keratin 17 was expressed in the peripheral corneal epithelium, and by 28 days it was expressed in the central cornea. PCNA, a proliferation marker, was constitutively expressed in the nuclei of control corneal epithelial cells. Three days post-SM, PCNA expression was decreased along the damaged basal layer. Twenty eight days post-SM, PCNA was upregulated in the nuclei of hyperplastic cells of the central cornea and cells of the limbus, indicating wound repair. These data suggest that limbal and /or conjunctival epithelial cells play a role in the repair of SM-induced corneal injury. A better understanding of the mechanisms of SM-induced corneal damage will lead to the development of new therapeutics for the treatment of these debilitating ocular injuries. *Supported by NIH grant AR055073.*

2664 Effect of Supersaturated Oxygen Emulsion on Corneal Injury from Chemical Warfare Agent Chloropicrin Exposure

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Ocular exposure to chemical warfare agents can lead to immediate pain, edema of eyelids, inflammation, massive corneal necrosis and blindness. Ocular chemical injuries have frequently been associated with poor prognoses, and the loss of viable corneal epithelial cells, leaving patients with few effective treatment options. It is evident that oxygen plays a vital role in ocular tissue preservation and is critical for tissue preservation and wound repair, especially in case of chemical injuries. This is important in avascular tissues like the cornea and delicate cell populations such as in the limbus. The oxygen therapy derives from a liquid *supersaturated oxygen emulsion* (SSOE) wound-healing technology originally funded by DARPA, and reformulated for ocular use. Oxygen levels in the emulsion and ocular tissues treated with the SSOE were evaluated using a micro-sensor. To test the efficacy of SSOE in treating corneal injury from chemical exposure, we used chloropicrin (CP). CP is a broad-spectrum pesticide that has been used in chemical warfare and causes severe ocular toxicity. Our team has previously shown that application of the SSOE (55%) to human corneal epithelial cells increased cell viability, wound (scratch) healing and reversed CP induced DNA damage, apoptotic cell death, and oxidative stress markers. In the present study, we have developed an *ex vivo* rabbit corneal injury model using CP. Corneas were exposed to CP (500 or 700 nmol) for 2 h, and then washed and cultured in media with or without supersaturated oxygen emulsion (SSOE; 55%) for 24 h or 96 h. Corneal sections were collected and hematoxylin and eosin (H&E) and immunohistochemical (IHC) stained as well as subjected to molecular analyses for apoptotic cell death, inflammatory markers like cytokines. SSOE treatment abrogated the CP-induced cell death and degradation at 24 h post exposure. At 96 h of corneal culture, SSOE treatment enhanced healing of the epithelial layer as well as reversed CP-induced keratocyte cell death. Ongoing molecular analysis will further help decipher the protective effects and mechanism of the SSOE in the *ex vivo* cornea culture injury model. Additional studies to examine the treatment efficacy of SSOE in reversing chemical injury or enhancing wound healing in *in vivo* corneal injury rabbit models are warranted.

2665 *In Vivo* Injury Models, Biomarkers, and Dexamethasone Treatment for Corneal Damage by Vesicating Agents

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Vesicating agents, Sulfur mustard (SM), Nitrogen mustard (NM) and Lewisite (LEW), are potent chemical warfare agents that primarily cause damage to the ocular, skin, and respiratory systems. Of these, ocular tissue is the most sensitive organ. Ocular vesicant exposure causes photophobia, corneal lesions, corneal edema, ulceration, and neovascularization, and may result in a complete loss of vision. The ocular injury upon exposure to SM, the most extensively used vesicant in warfare, has been well documented. NM is a bi-functional analogue of SM and has been shown to cause injuries similar to SM. However, there are very few reports on ocular injury by LEW. The injury symptoms upon LEW exposure appear instantly after exposure compared to SM. Despite extensive research efforts, effective therapies for vesicant-induced ocular injuries, mainly to the most affected corneal tissue, are not available. One of the major impediments to progress in the discovery and validation of ocular therapies against vesicating agents is the lack of established and accessible ocular injury models suitable for the studies on the pathogenesis of eye lesions and associated mechanisms. We have established useful ocular injury clinical, biological and molecular markers in *in vivo* rabbit corneal model to understand the mechanism of injuries from NM, SM, and LEW exposure. Using these markers as relevant end points, we have evaluated the efficacy of glucocortecosteroid dexamethasone formulation (DM) for treating vesicant-induced ocular injuries. DM exerts immunosuppressive and anti-inflammatory effects, and regulates proliferation and apoptosis in corneal epithelial cells. DM treatment resulted in a significant reversal in mustard and arsenical vesicants-induced ocular injury parameters. DM treatment reduced vesicants-induced clinical and biological markers including corneal opacity, corneal neovascularization and corneal thickness. DM treatment also reduced vesicants-induced COX-2, VEGF, and MMP-9 expression. Together, these studies warrant further *in vivo* optimization of DM treatment in mustard and arsenical vesicants-induced corneal injury and to understand the mechanism of efficacy.

2666 Nitrogen Mustard Targets Pathways for Tetrahydrobiopterin Biosynthesis in Human Keratinocytes

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Nitrogen mustard, mechlorethamine (bis(2-chloroethyl)methylamine; HN2), and sulfur mustard are potent vesicants that can modify cellular macromolecules and disrupt metabolism. Important in many metabolic processes including endothelial function, cell growth regulation, neuropathic pain, oxidative stress, immunity and inflammation, is tetrahydrobiopterin (BH4, 6R-L-erythro-5,6,7,8-tetrahydrobiopterin) a key cofactor for nitric-oxide synthases as well as aromatic amino acid hydroxylases important in synthesizing catecholamines, neurotransmitters, and indoleamines. We have discovered that HN2 targets the BH4 biosynthetic pathway in HaCaT cells, a human keratinocyte cell line. BH4 is generated in HaCaT cells from dihydrobiopterin (BH2) via the enzyme NADPH sepiapterin reductase using sepiapterin as a substrate. Once formed from sepiapterin, BH2 is converted to BH4 by various intracellular reductases including dihydrofolic acid reductase. Treatment of HaCaT cells with HN2 for 1-4 hr was found to cause a concentration-dependent inhibition of intracellular biosynthesis of BH2 and BH4 (IC_{50} = 28.5 μM) without affecting cell viability. Inhibition of BH4 biosynthesis was not due to inhibition of the reduction of BH2. Thus, HaCaT cells pretreated with HN2 (10-160 μM) readily synthesized BH4 when incubated with BH2. In cell lysates, incubation with sepiapterin (200 μM) readily generated BH2. Treatment of cell lysates with HN2 caused a concentration-dependent inhibition of BH2 formation (IC_{50} = 31.6 μM) indicating that the vesicant was targeting NADPH sepiapterin reductase. Using human recombinant NADPH sepiapterin reductase, we found that HN2 caused a time- and concentration-dependent inhibition of enzyme activity (IC_{50} = 74.6 μM). Taken together, our data demonstrate that HN2 targets BH2/BH4 biosynthesis in human keratinocytes by targeting NADPH sepiapterin reductase. Overcoming inhibition of this enzyme by HN2 will be important in ameliorating toxicity of HN2 associated with enzymes that are dependent on BH4. *Supported by NIH grants AR055073 and ES005022.*

2667 Optimizing Nitrogen Mustard Administration to Obtain Reproducible Model Vesicant Skin Burns

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Sulfur mustard (SM) is a chemical warfare vesicant that damages exposed skin, eye, and lung tissues requiring the advancement of countermeasures to develop effective therapies. Nitrogen mustard (NM) is a primary vesicating and bifunctional agent widely used as a model vesicant surrogate for SM owing to its comparable skin injuries and similar mechanisms of cytotoxicity. A consistent NM skin injury/burn animal model is required for wound healing efficacy studies. Researchers have established consistent NM burn efficacy models with mixed levels of success. Our currently used model entails shaving the dorsal skin of female CD-1 mice at the lumbar region and delivering a 20 μmol bolus of NM in acetone to the skin using 6mm glass fiber filter discs as a wound template. The NM induced skin inflammation was evaluated by punch biopsies (on day 3) of the treated area and compared to the control groups. Although this model resulted in comparable wound severity, we could not obtain highly reproducible NM skin burns. Therefore, we hypothesized the variation was related to hair shaft height, follicle density, sequence of acetone/NM administration and dose/volume delivered. The first hypothesis tested was that shaving hair resulted in variable contact between the skin surface and filter discs. To improve contact between the skin and filter discs, a chemical depilatory, Nair™, was employed to remove the hair from the epidermal surface. Results indicate that hair removal using the depilatory appreciably reduced burn incidence inconsistencies as compared to the shaving method. After shaving, the hair shaft was present above the entire epidermis and stratum corneum, while the application of the depilatory shortened the hair shaft to beneath the epidermal surface and preserved the follicle. Histological analysis revealed improved contact surface using Nair™ with no significant alterations of skin structure. In addition, it was found that by changing the sequence, volume and concentration of acetone/NM administration, model burns could be reliably and consistently produced yielding a model that allowed for countermeasure efficacy studies in rodents. *Supported by NIH grant #U54AR055073.*

2668 Treatment of Sulfur Mustard Burns: Silvadene, Silver Nylon, or Combined and Evaluation of Silver Ion Distribution in a Minipig Model

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Sulfur mustard (SM) is a chemical weapon / terrorist weapon that is easily synthesized, concealed and deployed. SM skin exposure results in burns that are indolent, refractory and recurrent. We tested silver-nylon (Silverlon® BCD) dressings, silver sulfadiazine (SSD), and a combination of both in a porcine model of partial thickness SM injury as a GLP non-inferiority study. A total of 680 deep partial thickness burns were produced in 85 Gottingen minipigs by vapor cap exposure for 90 minutes. Wounds were then debrided with 7 days of saline wet/wet dressings. BCD dressings were applied and changed either daily, or every 4 or 7 days. Control SSD dressings were changed daily. A cohort of 5 animals were treated with SSD placed underneath Silverlon® and changed daily. Dressings were applied for 30 days followed by 7 days of no treatment. Animals were euthanized and wounds assessed for complete healing and re-epithelialization by three subject matter experts. Secondary endpoints included body weight, food intake, normal behavior, and silver levels in wound and blood. There was no morbidity or mortality, or wound infection seen. All animals demonstrated normal behavior, appetite and weight gain. All wounds healed well and there were no appreciable differences in healing between the SSD + Silverlon® group and the others. Blood levels of silver were at or below lower limit of detection (0.03 µg/gram) in virtually all cases. Silver ion was detected in all wounds. Mean silver levels for 1, 4, or 7 day BCD treatment were 3.61, 5.43 and 4.4 µg/g respectively. Mean silver levels for SSD were 3.74 µg/g. Mean wound sliver levels for BCD combined with SSD were 2.84 µg/g (range 1.0 -11.0 µg/g). All treatment methods produced good wound healing, negative blood silver assay and elevated wound silver levels. SSD applied under silver-nylon dressings showed no advantage compared to the other methods and the combination of SSD with BCD had no deleterious effects to the wound or the minipig.

2669 Silver-Nylon Treatment of Sulfur Mustard Burns: Bioengineering Assessment of Successful Wound Closure

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Sulfur mustard (SM) is a chemical weapon first used in 1917 Ypres with 11 subsequent regional conflicts to include ISIS use in 2016 Syria. We tested silver-nylon (Silverlon BCD) dressings in a porcine model of partial thickness SM injury in a GLP non-inferiority study. A total of 1320 superficial (SD) or deep partial (DD) thickness burns were produced in 165 Gottingen minipigs by vapor cap exposure. The wounds were then debrided with 7 days of saline wet/wet dressings. BCD dressings were applied and changed either daily, or every 4 or 7 days. Control silver sulfadiazine (SSD) dressings were changed daily. Dressings were applied for 30 days followed by 7 days of no treatment. Animals were euthanized and wounds assessed by histopathology. The non-inferiority margin for histopathology score was 1.5 (10% of maximum possible score) when comparing BCD dressings to SSD. Secondary endpoints included body weight, wound closure, planimetry, Modified Draize Score (MDS), clinical chemistry, hematology, silver ions in selected tissue, colorimetry, and transepidermal water loss (TEWL). BCD, when applied once every 7 days to superficial or deep wounds, was not inferior to the comparator treatment, SSD, as evaluated by histopathology. Blood silver levels were drawn pre-, mid-study, and at necropsy in addition to liver, spleen and kidney samples and were negative with BCD, while with SSD silver ions were detected. Silver ions were detected in virtually all wounds with either treatment. The overall mean net wound color change was not different in the DD or SD with BCD or SSD by the end of the study. Planimetry also showed that while the eschar came off earlier in the SSD group, all wounds were close to 100% re-epithelialization by the end of the study. MDS were also unremarkable in that there was very little edema with the SM wounds; erythema and necrosis developed during the wound/injury phase and during the wet/wet saline debridement with complete resolution by the end of the treatment period and study. There were no significant differences in TEWL when comparing any of the treatment groups. Silverlon BCD dressings provide effective treatment for SM burns. This study and program work resulted in the first FDA clearance of Silverlon BCD bandage for a vapor sulfur mustard indication.

2670 Inhibition of Matrix Metalloproteinase 9 (MMP9) Suppresses Inflammation and Enhances Wound Healing in Mouse Skin Treated with Sulfur Mustard

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Sulfur mustard (bis(2-chloroethyl) sulfide, SM) is a highly reactive bifunctional alkylating agent that can induce inflammation and blistering in the skin. Effective medical countermeasures against SM-induced cutaneous injury have yet to be established. Overexpression of MMP9 has been implicated in the pathogenesis of SM-induced skin injury. MMP9 is known to degrade basement membrane (BM) and play a role in regulating the migration of macrophages and neutrophils to sites of tissue injury; inhibition of MMP9 reduces inflammatory cell migration and suppresses inflammation in tumors and various wound models. In the present studies, we tested ([N-hydroxy-3-phenyl-2-(4-phenylbenzenesulfonamido) propanamide], BiPS), a specific MMP9 inhibitor, for its ability to suppress inflammation and enhance wound healing following SM exposure using the mouse ear vesicant model. Treatment of male CD1 mouse ear skin with SM (0.08 mg) caused a characteristic cutaneous injury including edema, inflammatory cell infiltration, and the formation of microvesicles at the dermal-epidermal junction (DEJ). Expression of MMP9 mRNA and protein in the skin progressively increased with time 24-168 h following SM exposure. Immunofluorescence studies showed disruption of the BM molecule collagen IV (ColIV), increased expression of COX2, and upregulation of the skin wound marker keratin 6 (K6) in SM induced skin wounds. SM also caused macrophage (F4/80) accumulation in the tissue which persisted up to 168 h post exposure. Pretreatment of ear skin with BiPS reduced SM-induced dermal edema by 60% and maintained the integrity of the DEJ as evidenced by contiguous expression of ColIV. At 168 h post SM exposure, mRNA and protein expression of MMP9 was significantly downregulated in BiPS treated mice. BiPS treatment also suppressed SM-induced inflammation, reducing the number of macrophages accumulating in the tissue; it also suppressed expression of COX2 and downregulated expression of K6. These data indicate that targeting MMP9 by BiPS effectively protected mouse ear skin from SM induced injury. MMP9 inhibitors may be useful as medical countermeasure against SM-induced cutaneous injury. *Supported by NIH grants ES005022 and AR055073.*

2671 Nitrogen Mustard Induces Mitochondria-Mediated Apoptosis Associated with Endoplasmic Reticulum Stress-Regulated MAPK Signaling in Human HaCaT Keratinocytes

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Vesicating agents, including sulfur mustard (SM, bis(2-chloroethyl) sulfide) and nitrogen mustard mechlorethamine (HN2, bis(2-chloroethyl)methylamine), are bifunctional alkylating agents that are highly reactive in the skin causing extensive tissue damage and blistering. Previously, we reported that treatment of HaCaT human keratinocytes with HN2 caused a block in the S phase of the cell cycle and cell cycle dependent DNA damage signaling, processes contributing to cytotoxicity. In the present studies, we evaluated molecular mechanisms underlying HN2 toxicity. Multi-color flow cytometric analysis revealed that HN2 treatment caused a time- and concentration-dependent induction of apoptosis. This was associated with selective activation of caspase 3, caspase 6, caspase 7 and caspase 9, but not caspase 8 or caspase 10, suggesting that HN2 triggers mitochondrial-mediated apoptosis. The is supported by findings that HN2 also caused increases in levels of Bax and p62, and decreases in Bcl-xL. In contrast, HN2 had little or no effect on expression of LC3B and Beclin-1, markers of autophagy. Expression of cleaved caspase 3 and cleaved PARP was found to be homogenous throughout the cell cycle, suggesting that HN2-induced apoptosis is cell cycle independent. HN2 also triggered endoplasmic reticulum (ER) stress and activation of mitogen-activated protein kinase (MAPK) signaling in HaCaT cells, which was identified by enhanced phosphorylation of several key molecules including eukaryotic initiation factor 2α (eIF2α), p38, ERK1/2 and c-Jun N-terminal kinases (JNKs) and up-regulation of ATF4 expression. GSK2606414, an ER stress inhibitor, reduced phosphorylation of these proteins. Conversely, MAPK inhibitors, including SB203580, PD98059 and SP600125, blocked phosphorylation of p38, ERK1/2 and JNKs, but not eIF2α. These data indicate that ER stress regulates MAPK signaling in HN2-treated HaCaT cells. A pan-caspase inhibitor, z-VAD-FMK, was also found to suppress HN2-induced ER stress and MAPK activation and to attenuate cytotoxicity and block apoptosis. Taken together, these data demonstrated that ER stress and MAPK signaling play an important role in HN2-induced apoptosis, contributing to vesicant-induced cytotoxicity and tissue injury. *Support: NIH grants AR055073, NS108956, and ES005022.*

2672 Characterization of Molecular Pattern in Sensory Pain Signaling and Its Neurotransmitters on Mouse Skin following Mustard Exposure

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Sulfur mustard (Sulfur mustard (SM, bis(2-chloroethyl sulfide))) is a warfare chemical which causes inflammation, epidermal blistering, and the characteristic but the unique effect of delayed skin pain. However, the underlying mechanisms mediating sensory pain following mustard exposure remain unclear. We have previously demonstrated activation of cannabinoid signaling following exposure of skin to mustards. Therefore, we hypothesized that downstream signaling following activation of cannabinoid receptors by mustards may modulate the sensory pain following mustard exposure in the skin and mediate the 4-12 hour delay in pain often experienced. In the present study, we investigated reprogramming of dermal sensory pain receptors by chemokines following mustard exposure utilizing mouse skin models. In these studies, we investigated correlations between cell proliferation and cellular responses important in pain, we measured differences in cell proliferation and pro-inflammatory gene expression in keratinocytes following mustard exposure. Mustard exposure mediated a distinct pattern of cell proliferation (PCNA mRNA) that was altered in a time-sensitive manner; in contrast, mRNA levels for Keratinocyte marker protein (Krt10) following mustard exposure, exhibited distinct redox patterns. A four-fold induction NQO1 and NQO2 including GST-1beta expression in in the early phase was observed. Contrarily, the pattern of NOXs (NOX1, NOX4 and NOX5) expression in response to SM was upregulated by two or three-fold in the later phases (at 48hr) compared to early (24 hr) responses. Of note, the tropism of pain receptors in mouse skin was distinctly altered from kappa (OPRK) to delta opioid receptor (OPRD) in a time-dependent manner. In addition, we found that NGF mRNA may play a role in the early phase responses in contrast to other neurotransmitters including BDNF, GDNF, and PDYN. Interestingly, SM triggered transient receptors by up- /down- (to TRPV1 mRNA) or down-/up- (TRPV3 mRNA) in a time-dependent manner. In general, these results indicate that sensitivity to Cannabinoid receptors in mouse skin was diminished in later time periods when compared to early responses. We speculate that those opioid receptors may implement cannabinoid signaling by promoting TRPV1 signaling, a process effected by mustard exposure dependent manner in the dermal lesion.

2673 BRD4-Dependent Chromatin Remodeling by Vesicant Arsenicals in Murine Skin

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Warfare or industrial chemicals are highly toxic and pose significant threat to the environment and human health if exposed accidently or deliberately. Arsenicals are warfare chemical vesicants, developed for use in World War-I/II. Skin exposure to these chemicals cause painful inflammation, however, the underlying mechanisms remain undefined. In other systems, various inflammatory responses have been associated with the remodeling of nuclear chromatins. In this regard, a role of bromodomain and extra-terminal domain (BET) family proteins has recently been highlighted. Bromodomain 4 (BRD4) mediated epigenetic regulation can be important in acute inflammatory responses. Here, we demonstrate histone lysine hyper-acetylation of H3 and H4 target proteins H3K9ac, H3k18ac and H3k27ac and H4k5ac and H4k8ac respectively following topical exposure to various arsenicals including lewisite, diphenylchlorarsine, diphenylcyanoarsine and diethylchlorarsine *etc.* in murine model. We show that arsenicals augment protein lysine acetylation. This is accompanied by the enhanced expression of BRD4. BRD4 regulated inflammatory genes such as SAA3, LCN2, FPR-1, SLFN-1, IL-6, and IL-19 are significantly-induced in the skin of arsenicals treated animals. Interestingly, these pro-inflammatory gene profile is identical for structurally distinct arsenicals, although the magnitude of induction differed. Furthermore, employing human skin keratinocytes and murine peritoneal macrophage, we demonstrate a cell context-dependent responses of BRD4-regulated inflammation. In this regard, chemokines such as CXCL1 and CXCL2 were predominantly altered in macrophage but not in skin keratinocytes. Our findings suggest that BRD4 is an initial central regulator of arsenicals-mediated skin inflammatory responses and could be a potent druggable target for developing antidotes against these and similar chemicals.

2674 Toxicologic Pathology and Related Mechanism following Cutaneous Exposure of Phosgene Oxime

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Phosgene Oxime (dichloroform oxime; CX), an urticant categorized as a vesicating agent, is a potential chemical warfare and terrorist agent. Its exposure causes rapid toxicity, severe pain with dermal symptoms including erythema (redness), urticaria, blanching, itching hives, necrosis and systemic effects. Due to its fast penetration, severe dermal injury, and instant lethal toxic effects resulting in high mortality, CX could be weaponized with other chemical warfare agents to enhance their deleterious effects. CX is one of the least studied vesicating agents with no effective treatment available. In the present study, we exposed the dorsal skin of SKH-1 hairless mice with neat CX for 0.5 and 1.0 min using two 12 mm vapor caps, and studied different parameters associated with its pathophysiology. Clinical data from our study showed that CX exposure leads to acute skin lesions (edema, erythema, necrosis, urticaria and blanching) as well as decreases in heart and respiratory rate, drop in body temperature, vasculature dilation and blood congestion in multiple organs indicating urticaria and anaphylaxis. Histopathological analysis showed a CX-induced increase of over 2-fold in the mouse skin epidermal thickness at 2 hr and 14 days following 0.5 min and 1.0 min exposures, respectively. Similarly, 1.5-2.0-fold increase in dermal plus hypodermal thickening was observed at all study time points following 0.5 and 1.0 min CX exposures. Dermal fibrosis, necrotic blood vessels, hyperkeratosis, cell death and an increase in inflammatory cells was also observed at day 1 and 14 following 0.5 min CX exposure. Edema, necrosis and dying fat cells were observed within 2 hr in the skin of both the CX exposure groups. CX induced an increase in mast cell degranulation within 30 min of its exposure which peaked to over 90% at 24 hr post exposure and was associated with increased histamine and tryptase levels. To further assess the observed CX-caused pathological changes, skin sections are currently being analyzed for macrophages (F4/80), apoptotic cell death (TUNEL staining) and myeloperoxidase activity (neutrophil infiltration). Molecular markers and cytokines associated with CX-induced mast cell degranulation and inflammatory responses are being evaluated to identify the signaling pathways and key molecular targets in toxicologic pathology from CX cutaneous exposure.

2676 Development of an Organophosphorus Skin Disclosure Kit for Whole Body Surface Sampling

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The recent use of nerve agents with low vapor pressure and high percutaneous toxicity such as VX and Novichok for assassinations in Malaysia and the UK underline the constant threat by these compounds for both the military and civilian chemical defense. Early disclosure and detection are key issues in the management of chemical casualties. However, a major gap exists for nerve agents with low vapor pressure and high percutaneous toxicity such as VX and Novichok. To overcome this obstacle we recently developed a robust but sensitive, easy to use and generic organophosphate skin disclosure kit. A moistened cotton swab is used for skin sampling and put into a test tube pre-filled with test buffer. Lyophilized erythrocyte membranes as acetylcholinesterase (AChE) source dissolved in the buffer of the test tube allow incubation with the samples from the swab and AChE. Subsequently, lyophilized acetylthiocholine and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) are added to facilitate Ellman reaction. Formation of a yellow end product advocates absence of AChE inhibitors including nerve agents, pesticides and carbamates while colorless or pale yellow indicates the presence of an AChE inhibitor. The whole procedure takes only about 5 min and can be performed easily in the field. It showed a lower limit of detection with V-type nerve agents on glass slides in the range of 100 ng and 10 µg with the OP pesticides paraoxon-ethyl and malaoxon being several orders of magnitude lower than estimated incapacitating or lethal doses of the respective compound. However, the skin area to be detected with the field test is very limited whereas already µL-amounts of nerve agent somewhere on the whole body surface of humans might pose a lethal dose. Thus, we developed a scaled up kit comprising a sponge fixed to a customized holding device and a small bucket including chemicals for Ellman reaction. The kit allows whole body sampling and identification of nerve agent-exposed people without or with unclear clinical signs in need of decontamination and medical countermeasures within 2 min. When 800 cm2 porcine belly skin were sampled 5 min after exposure to 2 µl VX with

the whole-body OP disclosure kit, the test results unambiguously advocated presence of an AChE inhibitor. The whole-body OP disclosure kit might be a helpful tool in disaster and military medicine for the identification of victims exposed to a broad range of AChE-inhibiting agents and rationally allocate limited medical countermeasures (e.g. decon, specific antidotes).

PS 2677 Biomarkers of Chlorine Gas Exposure in a Swine Model

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Chlorine (Cl_2) gas has been used as a chemical weapon since World War I and most recently in the Syrian conflict. In the United States, transportation and industrial accidents have caused multiple fatalities. Cl_2 exposures cause severe injuries to the respiratory, cardiovascular and nervous systems. However, biomarkers that specifically indicate Cl_2 exposure remain to be identified and validated. Previous studies in rodent models demonstrated that inhaled Cl_2 reacts with plasmalogens to form chlorinated aldehydes and fatty acids, detectable in plasma and lung tissue upon Cl_2 exposure. It is unknown whether these biomarkers are also formed in Cl_2-exposed large animals such as swine, a species used as a translational model for human exposures, and for FDA-enabling studies to identify Cl_2 injury countermeasures. Here, we examined whether chlorinated fatty acids can be detected in Cl_2-exposed pigs. Specific pathogen-free Yorkshire swine were exposed to Cl_2 gas (≤ 240 ppm for 1 h) or filtered air, under anesthesia. Plasma samples were collected at various time points and lung tissues were collected immediately after euthanasia. Total (free+esterified) 2-chlorofatty acids were measured by LC/MS after base hydrolysis, while free 2-chlorofatty acids were not subjected to base hydrolysis. Extractions were performed using plasma fortified with 2-chloro-[d4-7,7,8,8]-palmitic acid (2-[d4]-ClPA) as internal standard using Dole extraction. Lung tissue was pulverized and lipids extracted using the Bligh-Dyer method in the presence of 2-[d4]-ClPA. Half of the lung extract was subjected to LC/MS analyses of free 2-chlorofatty acids, and the other half subjected to alkaline hydrolysis followed by LC/MS for total 2-chlorofatty acids. Exposure to Cl_2 gas resulted in severe hypoxemia, increased airway resistance and peak inspiratory pressure, lung neutrophil infiltration and decreased dynamic lung compliance. Vascular leakage and edema were evident in Cl_2 exposed pigs, and increased levels of pro-inflammatory cytokines. Free- and esterified 2-chloropalmitic acid (2-Cl-PA) and 2-chlorostearic acid (2-Cl-SA) were detected in the lungs and plasma of Cl_2-exposed pigs, but not in clean air-breathing controls. Levels of Cl-lipids in plasma were highest immediately after Cl_2 exposure, and then declined with levels remaining 40- to 60-fold higher at 24h compared to baseline. Free- and esterified 2-Cl-PA and 2-Cl-SA were also significantly increased in the lungs of Cl_2 exposed-pigs. These observations in swine models validate chlorinated fatty acids as biomarkers of Cl_2 exposure across species, potentially serving as forensic biomarkers to validate human exposures.

PS 2678 Investigating the Role of Mast Cell Activation by Nitrogen Mustard in Lung Toxicity

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Chemical warfare agents (CWA) were widely used during the Gulf War (1990-1991) and as a result left ~30% of veterans suffering from Gulf War Illness (GWI). The vesicating agent, sulfur mustard (SM) is one of the most noteworthy CWA used during this time and exposure results in similar symptoms as those seen in GWI. Prior research has demonstrated that SM targets the bone marrow and has the potential to influence immune cells. Previous data in mice exposed to SM demonstrated a significant increase in pro-inflammatory cytokines followed by infiltration of neutrophils and macrophages in the lung. In the skin, exposure to SM was reported to induce mast cell degranulation. Therefore, the aim of this study was to determine if nitrogen mustard (NM) (a surrogate for SM) exposure promotes activation of mast cells. Using murine bone marrow derived mast cells (BMMCs) we assessed early, intermediate and late phase activation through degranulation, eicosanoid expression, and pro-inflammatory cytokine production respectively. While NM exposure (1 μM – 50 μM) did not result in mast cell degranulation, we observed an increase in intermediate phase activation demonstrated by a 30-fold increase of cyclooxygenase-2 (COX-2) mRNA production at 10μM at 6h. In addition, IL-6 production by BMMCs post 24h exposure to NM demonstrated a dose dependent increase from 10μM - 50μM, indicative of late phase activation. To further demonstrate a pivotal role for mast cells in NM exposure, we compared the effects of NM exposure on lung pathology between C57BL/6 and B6.Cg-KitW-sh/HNihrJaeBsmJ (mast cell deficient) mice. Significant lung injury was observed in C57BL/6J following NM exposure at 72 hrs as indicated by increase immune cell infiltration as well as red blood cell accumulation in the alveolar sacs. In contrast, significantly less injury was observed in mast cell deficient mice. Similarly, increases in total protein and IL-6 was observed in C57BL/6 mice but not mast cell deficient mice. These results suggest that mast cells play a prominent role in the injury induced by NM and may contribute to symptoms observed in GWI.

PS 2679 Differential Activation of Alveolar Type II Epithelial Cells and Alveolar Macrophages after Nitrogen Mustard Exposure in Rats

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Nitrogen mustard (NM) is a cytotoxic vesicant known to target the lung, causing acute injury which progresses to fibrosis. In previous studies, we demonstrated that alveolar macrophages (AM) are activated following NM exposure and contribute to the pathogenic response. In the present studies, we assessed the effects of NM on alveolar Type II (TII) epithelial cells. Male Wistar rats (150-174 g; 8-10 weeks) were euthanized 3 days after intratracheal administration of NM (0.125 mg/kg) or PBS control. TII cells and AM were collected 3 d later. NM exposure resulted in increases in bronchoalveolar lavage (BAL) cells, protein, IgM, receptor for advanced glycation end products (RAGE), high-mobility group box (HMGB)-1 and surfactant protein (SP)-D; AM and TII cells also increased, indicating damage to the alveolar epithelium and inflammation. This was associated with increased numbers of microparticles in BAL, suggesting activation of inflammatory and cell death pathways. To assess this, we analyzed expression of proteins associated with these pathways in AM and TII cells by western blotting. Expression of inflammatory proteins SP-D, the C-type lectin Dectin-1 and RAGE increased in AM, but not in TII cells after NM exposure. The pro-inflammatory protein, matrix metalloproteinase-9 was also increased in AM, as well as TII cells after NM exposure, while HMGB-1 was decreased. The PI3K/Akt and suppressor of cytokine signaling (SOC)-2 pathways are known to regulate inflammation. NM exposure downregulated expression of PI3K, Akt and SOC-2 in TII cells, with no significant effects in AM. Expression of pro-apoptotic proteins caspase-3 and caspase-9 increased in AM and TII cells, respectively, after NM exposure. In contrast, NM down regulated expression of the pro-apoptotic protein, poly-ADP-ribose polymerase (PARP) in TII cells, with no major effects in AM. Significant increases in the anti-apoptotic protein, Mcl-1 were observed in AM after NM exposure, but decreases in the anti-apoptotic protein, β-catenin was noted in TII cells. These data demonstrate that TII cells, like AM, are highly responsive to NM, and that distinct inflammatory and cell death signaling pathways are activated in these two cell types in rat lung. These findings suggest that TII cells and AM play unique roles in the pathogenesis of pulmonary toxicity caused by mustards.
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PS 2680 Pulmonary Effects of Inhaled Sulfur Mustard in Rats

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Sulfur mustard (SM, 2 (bis-chloroethyl) sulfide) is a cytotoxic vesicant used as a chemical warfare agent known to target the lung. Herein, we characterized the progression of SM-induced pulmonary injury in rats. Male Wistar rats were anesthetized, intratracheally intubated, and exposed to 0.4 mg/kg SM by vapor inhalation. Animals were euthanized 3, 7, 16 and 28 d later. SM inhalation resulted in necrosis of proximal bronchiole epithelium, peribronchial edema and increases in inflammatory cells 3 d post exposure. BAL levels of fibrinogen, matrix metalloproteinase (MMP)-9, receptor for advanced glycation end products (RAGE), and surfactant protein (SP)-D were also increased at 3 d, consistent with alveolar epithelial cell injury. This was correlated with increases in BAL cell number, protein content, and levels of high mobility group box (HMGB)-1. SM also induced oxidative and nitrosative stress in the lung, as measured by expression of heme oxygenase (HO)-1 and inducible nitric oxide synthase (iNOS). Lung levels of proliferating cell nuclear antigen (PCNA) and fibrinogen were also upregulated at 3 d post SM, along with tumor necrosis factor (TNF)α, cyclooxygenase (COX)-2, MMP-9, galectin (Gal)-3 and Ym1. These inflammatory changes persisted in the lung at relatively lower levels up to 16 d post SM exposure when epithelial damage and peribronchiolar inflammation were also evident in distal and respiratory bronchioles. At 28 d post SM, BAL cells and protein, as well as MMP-9, RAGE, SP-D and fibrinogen were increased to levels at or above those observed 3 d post SM. In addition, at 28 d post SM, PCNA, MMP-9, iNOS, COX-2, fibrinogen, Gal-3 and Ym1 were also upregulated. This correlated with the appearance of airway protein-

aceous exudate entrapping inflammatory cells, diffuse squamous metaplasia, aberrant bronchial epithelial repair and multifocal alveolar interstitial and peribronchial fibrosis. These data demonstrate that similar pulmonary pathologic events occur in rats and humans following SM exposure and suggest that this rodent model will be useful for mechanistic studies on SM and for the identification of efficacious therapeutics for mitigating acute injury and fibrosis. *Support: NIH U54AR055073, R01ES004738, P30ES005022; EUH 778051; MSHEP 3899/H2020/2018/2.*

2681 Microparticle Detection and Phospatidlyserine Exposure in Airway Surface Liquid in Response to Nitrogen Mustard Inhalation in Rats

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Inhalation of mustard alkylating agents injures the respiratory tract, resulting in airway inflammation and procoagulant effects. Evidence suggests that microparticles (MP) released following exposure to toxicants play a role initiating inflammation and coagulopathy. In these studies we determined if MPs are involved in the adverse effects of nitrogen mustard (NM). Sprague-Dawley rats were exposed by intratracheal instillation to vehicle (PBS) or NM (0.15 mg/kg). Twenty-four hours later, rats were euthanized and MPs were analyzed in bronchoalveolar lavage fluid (BALF). To identify MPs, BALF was fractionated into three distinct populations by serial centrifugation (Fraction 1: 1,200 *g*/10 min; F2: 10,000 *g*/30 min; F3: 108,000 *g*/60 min). Western blot analysis revealed high level mitochondrial aconitase expression in protein extracts recovered from the initial two fractions, whereas the vesicular sorting protein ALIX, an exosomal marker, was detected almost exclusively within the third fraction. Flow cytometric analysis revealed a three-fold increase in MPs (0.15-1.0 µm) in all three fractions following NM exposure. Use of a lactadherin-FITC probe to detect phosphatidylserine+ MPs also demonstrated increased percentages of positive events in initial and intermediate MP fractions from NM-exposed subjects. Despite increases in MPs and phosphatidylserine positivity, no change in the level of ALIX was observed between NM and control groups. Our findings indicate MPs are increased in BALF from rats exposed to NM. The increasing proportion of phosphatidylserine+ events in the absence of any change in ALIX suggests the rise in MPs originates from apoptotic events rather than increased exosome release by airway cells.

2682 Farnesoid X Receptor Regulates Macrophage Activation in Nitrogen Mustard-Induced Lung Injury in a Sex-Dependent Manner

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Nitrogen mustard (NM) is a cytotoxic vesicant known to cause acute lung injury which progresses to fibrosis. Following NM exposure, there is a sequential accumulation of pro-inflammatory/cytotoxic M1 and anti-inflammatory/wound repair M2 macrophages in the lung, which we have demonstrated contribute to lung toxicity. In these studies, we analyzed mechanisms regulating macrophage phenotypic activation, focusing on the dysregulation of lung lipids which we hypothesize promotes acute lung injury and fibrosis. Farnesoid X receptor (FXR) is a nuclear receptor involved in regulating lipid homeostasis and inflammation; FXR target genes have been shown to enhance anti-inflammatory M2 macrophage activity and metabolic programming. To analyze the role of FXR in macrophage activation, we used $FXR^{-/-}$ mice. Male and female WT and $FXR^{-/-}$ mice were treated with PBS (control) or NM (0.08 mg/kg, i.t.). Bronchoalveolar lavage (BAL) and lung tissue were collected 3, 14 and 28 days later. NM caused progressive histopathologic alterations in the lung including inflammatory cell infiltration, septal damage and epithelial thickening; increases in expression of heme-oxygenase-1 (HO-1), a marker of oxidative stress were also noted, along with elevations in BAL protein and cells, markers of alveolar epithelial injury. These changes were more prominent in $FXR^{-/-}$ mice at all times examined, especially in males. Additionally, in male, but not female $FXR^{-/-}$ mice, there was evidence of foamy macrophages and fibrosis, as assessed histologically and by Gömöri trichrome staining. NM-induced increases in pro- and anti-inflammatory lung macrophages as quantified by flow cytometry, were also more significant in male $FXR^{-/-}$ mice, when compared to female $FXR^{-/-}$ mice. These findings demonstrate that FXR modulates the response of macrophages to NM and is involved in regulating lung injury, oxidative stress and the development of fibrosis. Our observation that male mice lacking FXR are more sensitive to NM than female mice suggests a potential protective role of estrogen signaling in this model of lung injury. *Supported by NIH Grants AR055073, ES004738 and ES005022.*

2683 Evaluation of Multiple Dosing of Nucleic Acid Neutralizing Agent on Survival of Rats Exposed to an Analog of Sulfur Mustard

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Sulfur mustard (SM) is one of the major chemical warfare agents. Its acute/chronic exposure causes injury to the respiratory tract, eyes and skin leading to multi-organ damage. Among these, pulmonary toxicity appears to be the major cause of mortality. Our studies in rats exposed to 2-chloroethyl-ethyl sulfide (CEES, aka: half mustard), a SM analog, have implicated nucleic acid release as the underlying cause of CEES-induced activation of inflammatory and coagulation cascades, which lead to lung injury and death. Previously, we have shown that exposure of rats to aerosolized CEES caused around 60% mortality by 12 h. We also demonstrated efficacy of a single dose administration of hexadimethrine bromide (HDMBr), a nucleic acid neutralizing agent, in mitigating CEES-induced injury and survival (100% survival) in rats over a 12 h period after which they started to die. Here, we examine whether re-dosing of HDMBr can prolong survival of rats exposed to aerosolized CEES. Male Sprague Dawley rats were exposed to 10% CEES for 15 minutes via nose-only aerosol inhalation. HDMBr was administered 2 hours post CEES exposure and then every 10 hours thereafter. Re-dosing improved oxygen saturations as assessed by pulse oximetry. Re-dosing also improved arterial blood oxygenation. There was increase in PaO2 and a decrease in the PaCO2 in the HDMBr treated group. In the HDMBr treated group there was also decrease in protein leak in the lung as assessed by total protein and IgM levels in the bronchoalveolar lavage fluid. More importantly, re-dosing with HDMBr improved survival over a 48 h period. There was 78% survival in the HDMBr re-dosed animals compared to untreated group where there were no survivors. In the animals that received a single dose the survival was 20% in 48 h. Overall these results provide evidence for the improved efficacy of HDMBr upon repeat dosing and prolonged protection, highlighting the importance of nucleic acid neutralizing agents for treating CEES/SM induced lung injury.

2684 The Use of Molecular Imaging Methodology to Develop a Feed Forward Model to Assess Lung Function in Mustard-Induced Lung Injury

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Nitrogen mustard (NM; Tris[2-chloroethyl] amine) is a cytotoxic vesicant known to cause pulmonary damage which progresses to chronic fibrosis. Using computed tomography (CT) and magnetic resonance imaging (MRI) we can monitor NM-induced alterations in the lungs non-invasively as pathology develops. The goal of this study was to use structural changes identified by MRI and CT scanning to predict functional alterations, so as to conduct *in vivo* toxicological assessment. Male Wistar rats were exposed to PBS (control) or NM (0.125 mg/kg) via i.t. instillation. CT and MRI scans were performed prior to exposure and 3, 7, 14 and 28 d post exposure. To correlate pulmonary structure and function rats imaged at 3 and 28 d post NM exposure were assessed for lung function using a SCIREQ flexiVent. Baseline pulmonary mechanics were collected at a positive end expiratory pressure (PEEP) of 3 cm H_2O. Total lung volume was calculated from MRI (W) as it provides an accurate and artifact free assessment of lung tissue volume. The relative volumes of the lung using CT were used to summarize hyperinflation (I), normal lung, and consolidated lung tissue (B) using voxel density as a marker. CT is capable of differentiating between areas of the lung as air and water which are separated by over 1000 Hounsfield units; allowing voxel density to be used as a direct measure of fluid density within the tissue. Using these elements, we constructed a feed forward model to predict respiratory impedance (Z), a measure of airflow through a system, with the equation: $Z = ((H(\eta\text{-}j))/(\omega\text{^}\alpha))$ where $H = (B/(I+W))$. Calculated H revealed lung alterations following NM exposure (1.38 ± 0.08 n=4 in controls vs 0.99 ± 0.16 n=3) as a result of hyperinflation in NM-treated rats. The predicted Z spectra Z spectra generated using the flexiVent at PEEP 3. A strong correlation was observed between predicted and measured Z spectra at 28 d post NM ($R^2=0.98 \pm 0.01$, n=7). Both the predicted and the measured Z spectra were consistent with prior data showing loss of function at higher frequencies. Data was also analyzed at 3, 7, and 14 d to provide longitudinal analysis of the functional pathological changes over time. End expiratory pressure can be used to further assess lung function. These findings indicate that structural imaging data can be used to predict lung function as a toxicological endpoint following exposure to pulmonary toxicants. *Supported by NIH U54AR055073, R01ES004738, P30ES005022.*

2685 PADI2 Promotes Peribronchial Fibrosis in Phenyl Arsine Oxide-Exposed Mice

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The class of chemical warfare agents known as vesicants includes among others, arsenicals and mustard agents. Lewisite, the most common arsenical developed as a chemical weapon for use in World War II. Unfortunately, the stockpiles of Lewisite still exist. Lewisite causes deleterious inflammatory effects in the skin, and also manifest systemic damage including acute lung injury. However, survivors from arsenical exposure may develop chronic lung injury. The mechanisms underlying these effects are not known. Here, employing phenyl arsine oxide (PAO), an analog of Lewisite, we provide a novel mechanism that contributes to chronic lung injury following its cutaneous exposure. The mice were exposed topically to PAO and sacrificed 10 weeks later. The histological analysis demonstrated increased inflammation and the presence of peribronchial bronchitis. We observed an increased expression of PADI2 in the alveolar macrophages in PAO exposed mice. Our *in vitro* studies also demonstrate increased expression of PADI2 and ER stress-related proteins CHOP and ATF4 in macrophages treated with PAO. Additionally, these macrophages show increased production of pro-fibrotic mediators PDGF and TGF-b1. We used 'pulmospheres', an *ex vivo* 3D model of the human lung for this study. The incubation PAO activated macrophages with 'pulmospheres' results in increased invasiveness and myofibroblasts differentiation. The pharmacological and genetic inhibition of PADI2 results in decreased ER stress as well as secretion of pro-fibrotic mediators. Overall, these data demonstrate that PADI2 contributes to peribronchial bronchitis in PAO exposed mice. *This work was supported by NIH grant U54ES030246.*

2686 Cutaneous Lewisite Exposure Causes Acute Lung Injury in Mice

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Lewisite, an organo-arsenic compound first produced in 1918 is a strong vesicating and lethal warfare agent. Owing to its lipophilic nature, Lewisite can be readily absorbed through skin; thus, cutaneous exposure to Lewisite can make it immediately available to multiple organs via circulation. Previously, cutaneous exposure to Lewisite has been shown to cause skin lesions, multi-organ damage and death. A previous report showed decreased lung function in workers of a Lewisite factory. However, studies to characterize or address effects of cutaneous exposure to Lewisite on lungs are non-existent. To investigate whether cutaneous exposure to Lewisite would be injurious to lungs, we applied Lewisite at different doses (2.5, 5.0 and 7.5 mg/kg) to dorsal skin of age matched Ptch1$^{+/-}$/SKH-1mice. Twenty-four hours later, lung injury was assessed by measuring arterial blood gas parameters, neutrophils, cytokines and HMGB1 in bronchoalveolar lavage fluid (BALF). Moreover, lung tissues were assessed for histological scoring, cytokines and myeloperoxidase staining. A single topical application of each dose of Lewisite significantly increased partial pressure of CO_2 ($PaCO_2$) and decreased pH and partial pressure of O_2 (PaO_2) in arterial blood, implying impaired gas exchange. Histological evaluation and subsequent quantification showed an increase in lung injury score in Lewisite exposed mice. There was also a significant increase in neutrophils and other markers of lung inflammation like HMGB1, CXCL1 and CXCL5 in BALF 24 h post exposure. Similar increases in protein levels of CXCL1, CXCL5 and myeloperoxidases were also observed in the lung tissue of Lewisite exposed animals. These data collectively demonstrated that cutaneous Lewisite exposure causes acute lung injury in mice by increasing proinflammatory molecules and neutrophil infiltration.

2687 Utility of High-Throughput *In Vitro* Genotoxicity Methods for Hazard Identification and Categorization of "Data-Poor" Compounds

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The Toxicology Testing in the 21st Century (Tox21) consortium has used ~100 quantitative high throughput screening (qHTS) assays to acquire information on thousands of data poor compounds of environmental concern. Five of the Tox21 assays aimed to detect genotoxic potential of compounds. The MultiFlow DNA Damage assay is a multiplexed, high content, flow cytometry assay that classifies compounds as clastogenic, aneugenic, or non-genotoxic. As an orthogonal approach, we investigated whether MultiFlow could be remodeled as an HTS assay by using one time point (4 h) and a single, high concentration of test article. To evaluate this approach, we tested TK6 cells with a set of 49 reference compounds and generated unsupervised and supervised models for classification of compounds as active or inactive for genotoxicity. Compared to *a priori* calls, the new screen correctly classified 33/33 compounds as active and 13/16 as inactive. In a set of 84 NTP compounds previously evaluated using MultiFlow, we identified 46 compounds with sufficient data from conventional genotoxicity assays to be identified as clearly active or inactive. The new screen correctly identified 31/36 actives and 6/10 inactives. Of the 38 NTP compounds with insufficient genotoxicty data, 28 were active in both the new MultiFlow screen and the five Tox21 DNA damage qHTS assays. Retrospective analysis of standard MultiFlow data revealed that 17 of the 28 compounds were classified as genotoxic (clastogenic or aneugenic). Two additional analytical schemes were then applied to these data. First, benchmark dose was calculated for specific endpoints to enable potency ranking of the individual compounds. Second, the area under the curve was calculated to conduct unsupervised clustering to reveal relationships between the unknown compounds and the training set of well-understood genotoxicants. This comprehensive information collected across multiple assays contributes to more-informed characterization of data poor compounds and enhances decision-making around experimental design for additional testing requirements. The outcome of these experiments is the development of a combined-assay data generation and analytics strategy that can be scaled to much larger compound libraries and efficiently provide opportunities for read across-driven hazard identification and risk assessment.

2688 Effect of Age and Sex on Human Blood *PIG-A* Mutant and Micronucleated Reticulocyte Frequencies

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We previously described immunomagnetic separation/flow cytometry-based methods for enumerating two human blood biomarkers of DNA damage: micronucleated reticulocytes (MN-RET) and *PIG-A* mutant reticulocytes (mutant RET). Early work indicated that intra-subject variation of these endpoints is low and inter-subject variation is relatively high. We have now analyzed blood samples from 362 volunteers, the majority of which were self-reported healthy adults. Pediatric samples were obtained from several sources: cord blood from healthy full-term deliveries, babies prior to a diagnostic CT scan related to congenital abnormalities, and children being seen at a gastroenterology clinic. We found that mutant RET frequencies were influenced by age, sex, and age x sex interaction. For females, ANOVA showed each of the other 6 age groups exhibited significantly higher mutant RET than the youngest cohort (i.e., < 11 yr). Regression analysis indicated that the data were well described by a quadratic polynomial with an initial rise and a plateau (R^2 = 0.218; $p < 0.0001$). For males, ANOVA showed each of the other 6 age groups exhibited significantly higher mutant RET than the youngest cohort, as well as elevated frequencies for the 51-60 and 65+ yr groups compared to the 11-20 yr group. Regression analyses suggested the data were well described by a cubic polynomial that exhibited an initial rise, a leveling off, and finally an upturn (R^2 = 0.342; $p < 0.0001$). Statistical analyses showed no evidence for an age or sex effect on MN-RET values. These results suggest that while mutant cells accumulate with age, MN-RET do not. Further investigation into the factors that influence "spontaneous" mutant RET and MN-RET frequencies will be important for designing appropriately powered human biomonitoring studies.

2689 Weight of Evidence Assessment of the Genotoxicity of an Extract of Chinese Skullcap—A Traditional Chinese Herbal Medicine

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Chinese Skullcap (*Scutellaria baicalensis*) root has a long history of use in traditional Chinese medicine. It is widely used in dietary supplements marketed in U.S and one of the commercially available preparations is Chinese Skullcap powdered extract (CSPE). This specific extract contains a high level (~30 %)

of flavonoids, the most abundant of which are Baicalin and Wogonoside. To assess the genotoxic potential of CSPE, OECD guideline-compliant Ames and *in vitro* whole blood human lymphocyte micronucleus (HLM) assays were performed and found that CSPE was negative in the HLM, but weakly positive in the Ames test. As a follow-up to this finding, an OECD guideline-compliant HPRT study found CSPE negative for induction of gene mutation in mouse lymphoma L5178Y cells, suggesting that CSPE is unlikely to cause gene mutation in mammalian cells. It was hypothesized that CSPE flavonoids, like many other well-known botanical flavonoids, might cause genotoxicity through a secondary, threshold-like mode of action (MoA) like oxidative stress. In line with this hypothesis, CSPE tested negative in HLM in whole blood cultures, which can buffer oxidative stress, but increased micronucleus frequency in the human lymphoblastoid cancer TK6 cells. When TK6 cells were also tested in the presence of the antioxidant N-acetyl-cysteine, the effect shifted to higher doses at which intrinsic defense mechanisms were likely overwhelmed, further supporting the oxidative stress hypothesis. Likewise, in the Toxtracker® reporter assay, CSPE strongly activated gene expression markers for oxidative stress at doses lower than those inducing a weak increase in DNA damage markers, adding additional support for this hypothesis. In summary, the weight of evidence of the dataset for CSPE indicates that its weak mutagenicity observed in the Ames test was not reproduced in mammalian cells. Additionally, its *in vitro* clastogenicity observed in the TK6 cells is likely due to secondary effect via oxidative stress, which was further supported by its distinct dose-response profiles in Toxtracker® assays. Therefore, CSPE is unlikely to represent a genotoxic hazard, however, this assessment is specific to the particular preparation investigated.

PS 2690 Trifluoroiodomethane—*In Vivo* Transgenic Rodent Gene Mutation Assay

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Trifluoroiodomethane (CF_3I: CAS: 2314-97-8) is a non-flammable gas with several industrial uses, e.g., as a fire suppressant and a key component in refrigerants. CF_3I is positive for mutation in the Ames test (OECD TG471) and, at high inhalation exposures (>2%), it induces micronuclei in the bone marrow of exposed rats and mice. Due to its genotoxic profile, the EU classified CF_3I in 1997 as Cat. 2 Mutagen (i.e. substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans). To evaluate the *in vivo* mutagenicity of CF_3I, a transgenic rodent *in vivo* gene mutation assay (OECD TG488) in Big Blue® TgF344 rats was performed. This test provides a definitive measure of mutations *in vivo*. Male rats were exposed to CF_3I at 0, 0.125, 0.25 and 0.5% (0, 1250, 2500 and 5000 ppm) for 6 hrs/day (nose-only, inhalation), 7 days/week for 28 days. In addition to clinical observations, body weight, food consumption, and body temperature (measured hourly during exposure) were recorded. On Day 31, 3 days after the last exposure, lung (site of first contact), bone marrow (a high turnover tissue and the target for micronucleus induction), and liver (a slow turnover, control tissue) were analysed for *cII* transgene mutant frequency (MF). Inhalation exposure to CF_3I did not produce an increase in MF in either lung, bone marrow or liver. However, reduced body temperatures were observed in animals exposed to 0.25 and 0.5% CF_3I. More marked reductions in body temperature were observed in the preliminary phases of the study conducted at 1, 2 or 4% CF_3I. This is the first reported observation of body temperature effects following CF_3I exposure and provides a potential mechanism for formulation of micronuclei observed previously in rodents. Chemically induced hypothermia is known to induce bone marrow micronuclei via mechanisms that do not involve direct DNA reactivity (Tweats *et al*., 2007. Mut Res 627: 78-91). The data reported here confirm that the mutagenicity of CF_3I observed *in vitro* in the Ames test is not expressed *in vivo* in Big Blue TgF344 rats. Furthermore, the observation that CF_3I causes significant loss of body temperature in Big Blue TgF344 rats suggests the positive micronucleus findings seen in previous studies may have been caused by an indirect, non-genotoxic mechanism (i.e. hypothermia) rather than via direct DNA reactivity. Further work is being conducted to examine this hypothesis in more detail.

PS 2691 Investigation of Genotoxic Potential of Pharmaceutical Agents in the Alternative Chicken Egg Model (CEM)

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Genotoxic potential of a set of pharmaceutical agents with different pharmacological effects was assessed in the Chicken Egg Model (CEM) using comet and ^{32}P-nucleotide postlabeling (NPL) assays to detect DNA strand breaks and adducts, respectively. Among investigated compounds were a nonsteroidal anti-inflammatory drug (ibuprofen), antipyretic analgesics (phenacetin and its metabolite acetaminophen), local anesthetics (lidocaine and prilocaine), chemotherapy agents (cyclophosphamide and mitomycin C), antibiotic (ciprofloxacin) and antihypertensive vasodilator (hydralazine). Potential DNA damage was evaluated in the livers of chicken embryo-fetuses, which on days 9 through 11 of incubation received 3 daily injections of test substances at various dose levels and were terminated 3 hours after the last dose on day 11. Ibuprofen, phenacetin, acetaminophen and ciprofloxacin at the tested doses yielded negative results in the model, as expected based on the lack of their genotoxicity in other systems *in vitro* and *in vivo*. However, low solubility of these compounds limited the dose range tested in CEM. In contrast, several tested pharmaceutical agents yielded strongly or weakly positive outcomes in at least in one of the assays. For example, local anesthetics lidocaine and prilocaine produced DNA adducts which were chromatologically similar to those previously detected in rats by NPL technique. Additionally, lidocaine was positive in the comet assay, although the dose that induced DNA strand breaks was fetotoxic. Chemotherapeutic agents, cyclophosphamide and mitomycin C, which act as cross-linkers with established genotoxic potentials, also produced DNA damage in the model. Hydralazine which also previously showed evidence of genotoxic potential, produced DNA strand break, however, it was not dose dependent, and thus, this agent was considered to be equivocal in the CEM. In conclusion, DNA damage produced by several therapeutic agents requires additional investigation *in vivo* due to potential genotoxic properties.

PS 2692 Establishment of a Mutation Assay Using Whole Genome Sequencing of *Salmonella typhimurium* Cells

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Although next generation sequencing (NGS) provides a promising tool for mutagenicity studies on the detection and quantification of somatic mutations in the genomes of multiple cells, the technology is currently limited by the inability of NGS to distinguish somatic mutations from sequencing errors. Several approaches to sequence the whole genome of cells from single clones have been developed to address this problem. In this study, we evaluated the mutagenicity of several agents in *Salmonella* typhimurium TA100 cells using whole genome sequencing of single clones. *S. typhimurium* TA100, a commonly used Ames test strain, was treated with mutagens via two methods, continuous exposure in solid agar and short-term exposure in a liquid medium. Genomic DNA isolated from cells expanded from single clones in different treatment groups was sequenced and the data were analyzed using an established in-house bioinformatics pipeline to identify mutation frequency and mutation types. Our results showed that the mutation frequency was increased by the mutagens in a dose dependent manner. The major type of spontaneous mutations were G:C>A:T transitions, whereas the predominant types of mutagen-induc*ed mutations* reflected the "signature" mutations known for these mutagens. Moreover, the liquid medium exposure procedure increased the sensitivity for the test and minimized the sequencing coverage required for reliable mutation determination compared with the continuous exposure in solid agar. Taken together, our results suggest that the approach of using single-clone sequencing of the bacterial genome can be a practical, reliable, and cost-effective test method for detecting genome-wide somatic mutations.

PS 2693 Probiotic *Escherichia coli* Strain Nissle 1917—Preclinical Evaluation of Genotoxicity

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Escherichia coli strain Nissle 1917 (EcN) has been used as probiotic agent for more than a century. Among others, its probiotic effects were reported on diarrhea and inflammatory bowel diseases, with an efficacy similar to 5-aminosalicylic acid for the maintenance of remission in ulcerative colitis. EcN is well tolerated; however, *in vitro* DNA double-strand breaks were published suggesting genotoxic activity of EcN, related to colibactin. In order to evaluate the genotoxicity of EcN upon therapeutic treatment *in vivo* we initially estimated the MTD in male rats at 10^{11} CFU EcN/animal. This exceeds the daily maximum dose applied in human by a factor of 6×10^4. A study with oral administrations for 2 days was performed in 3 dose groups (10^{11}; 10^{10} and

10^9CFU EcN/animal). No clinical findings were observed. The genotoxicity was assessed by Alkaline Comet Assay. Cells from the small intestine, the upper and lower colon were isolated, embedded in agarose, lysed and DNA migration in electrophoresis was determined and expressed as tail intensity. Very interestingly, obtained tail intensities showed no biologically relevant differences in the dosing groups vs control. Next, the animals were treated daily p.o. with the same dose for 28 days. All animals survived with common back ground clinical findings. A histopathological evaluation of cecum, duodenum, colon, jejunum ileum, Peyer's Patches and rectum was performed. The EcN administration induced slight hyperplastic changes in Peyer's Patches and a minimal increased lymphocytes apoptosis at different doses in some EcN treated animals, which are related to the known immune properties of EcN. Most notably in the Alkaline Comet Assay no biologically relevant increase of tail intensity was found after treatment with the test item in cells from the small intestine as well as the upper and lower colon at any of the dose groups. The exposition with EcN was verified by assessment of feces samples taken from the animals before first application, on day 14 and on day 27 of the study. In summary these results suggest that in pre-clinical situation any EcN related genotoxicity is excluded and available *in vitro* data does not reflected this particular *in vivo* experimental setting.

2694 Risk Management of Topical Drug Development Candidates with *In Vitro* Aneuploidy Findings

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Aneuploidy risk management for oral drug candidates is well established. However, for topically applied compounds the approach is less defined because of a lack of readily available methodology to accurately measure unbound drug concentrations in proliferating skin keratinocytes of the stratum basale layer of the epidermis. In order to address this gap and develop tools to characterize aneugenic risk in skin, we explored several experimental approaches with previously reported aneugens. First, we evaluated the use of the 72-hour Epiderm™ 3D *in vitro* micronucleus assay for the detection and quantitative dose-response analysis for aneugenic agents with known mechanisms like tubulin binding and aurora B kinase inhibition administered both apically and in basal medium conditions. Second, we integrated mechanistic biomarkers that typically change with aneugenic response (phospho-H3, Ki67, polyploidy), which could be detected and quantified by immunohistochemistry in skin sections. Third, we compared the biomarker dose-response with the micronucleus dose-response using a benchmark-dose analysis (BMD) approach. The results from these initial studies show that the Epiderm™ micronucleus assay is sensitive for the detection of compounds that induce aneuploidy by either tubulin binding or the inhibition of aurora B kinase. The data further demonstrate that tubulin binders induce increases in mitotic cells as measured by Ki67 or phospho histone H3 at or below the concentrations where micronucleus induction is observed. By comparison, aurora B inhibitors induce decreases of mitotic cells and increases in polyploid cells at doses that are equivalent to the concentration were micronucleus induction is observed. The results indicate that the Epiderm™ 3D *in vitro* micronucleus assay could be used to risk manage early topical drug candidates. We conclude from the quantitative analysis that Ki67 changes (i.e. increase with tubulin binders and decrease with aurora inhibitors) might be applied as a surrogate marker in skin from dermal toxicity studies to evaluate aneuploidy risk following the topical application of compounds with *in vitro* aneugenic properties. Thus far our data support that histopathology biomarkers may be able to be used as surrogates for aneugenicity risk assessment in nonclinical dermal minipig studies, and future *in vivo* studies could be conducted to build confidence in this approach.

2695 Drug-Induced Replication Stress Drives Genome Instability in Mammalian Genomes

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Genome instability is a hallmark of most human cancers and is exacerbated following cellular exposure to drugs/xenobiotics that promote replication stress and low-fidelity DNA repair. However, the effects drugs/xenobiotics have in promoting genome instability in normal cells remains unexplored. Here, we studied the genomic consequences of drug-induced mutagenic DNA damage response (DDR) following treatment of Sprague Dawley rats with 10 mg/kg of cyclophosphamide for two, three or fourteen days followed by PCR-free whole genome sequencing (WGS) at a sequencing depth of approximately 30X of isolated bone marrow and duodenum cells. Drug-induced somatic variants/rearrangements were called using the Sentieon TNScope pipeline that was previously vetted by the Precision FDA truth challenge. Cyclophosphamide-induced chromosomal structural rearrangements including inter-/intra-chromosomal translocations, were evident in all treatment groups irrespective of the study duration. Cyclophosphamide induced somatic structural rearrangements at 99, 90, 82 genes in the bone marrow and 90, 89, 84 genes in the duodenum following 2-days, 3-days or 14-days of treatment, respectively. Inter- and intra-chromosomal translocations were evident in treated bone marrow and duodenum samples and impacted an average of 36 genes in each sample. Comparatively, somatic structural rearrangements were largely absent in all vehicle treated controls. Pathway level analyses revealed a subset of cancer-associated genes, including some tumor suppressors genes, to be mutated more frequently than expected by chance alone. In summary, assessing genomic endpoints has the potential to identify drug-induced genome instability (DiGI) in mammalian genomes, which is not adequately addressed by current testing standards.

2696 Unraveling the Role of UVA Exposure and Melanin in Melanomagenesis

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Cutaneous malignant melanoma (CMM) occurs mainly in sunlight-exposed skin. More than 70% of CMM cases have mutations in the oncogenic B-RAF gene, codon 600, all of which, intriguingly, lack the UV-induced pyrimidine-dimer signature. This study raises the possibility that DNA damage other than dipyrimidine photoproducts plays a crucial role in melanomogenesis and that B-RAF codon 600 is a preferential target. To test this possibility, we determined UVA-induced oxidative DNA damage (ODD) in melanocytes (MC) versus normal human skin fibroblasts (NHSF), and we mapped the ODD distribution at the nucleotide level in the B-RAF gene. We found that UVA induces higher levels of H_2O_2 and 8-oxo-deoxyguanosine in darkly pigmented African American MC (AAMC) than in lightly pigmented European American MC (EUMC), but induces neither H_2O_2 nor 8-oxo-deoxyguanosine in NHSF. Besides, UVA does not induce dipyrimidine photoproducts in MC or in NHSF in this study. UVA induces mutations, mainly thymine to adenine base substitutions, in MC, but does not induce mutations in NHSF. *In vitro*, UVA induces higher levels of H_2O_2 and 8-oxo-deoxyguanosine in a eumelanin solution than in a pheomelanin solution. Significantly, we found that the frequency of ODD formed at the purines in B-RAF codon 600 is 2-fold higher than at other sequences examined in MC. No such differences were found in H_2O_2-treated NHSF. We conclude that UVA sensitizes melanin to generate H_2O_2, which induces ODD and that the B-RAF codon 600 in MC is a preferential target. These results indicate that UVA plays a major role in initiating melanomagenesis.

2697 SPRTN Knock-Down Elevated the Level of Formaldehyde-Induced DNA-Protein Crosslinks and Impaired the BPDE-DNA Adducts Repair in the K562 Cell Line

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DNA-protein crosslinks (DPCs) are bulky DNA lesions formed with the covalently linked DNA-binding proteins and DNA, which can interrupt DNA replication and cause genome instability. Formaldehyde is a typical crosslinking agent causing DPC formation, but the repair mechanism of formaldehyde-induced DPCs is not well understood. SPRTN was recently proposed to be a key player in DPC repair. Whether SPRTN plays a role in the repair of formaldehyde-induced DPCs is unknown. As bulky lesions, DPCs can disturb the DNA replication, however, whether formaldehyde-induced DPCs affect the repair of other DNA adducts are still unclear. Here, we knocked down the expression of *sprtn* gene in K562 cells and investigated the formaldehyde-induced DPC formation. We first treated wild-type and *sprtn* knock-down cells with [$^{13}CD_2$]-formaldehyde for 2 hours, and quantified the formaldehyde-induced DPCs by ultra-performance liquid chromatography and high-resolution mass spectrometry (UPLC-MS). We found that *sprtn* knock-down cells had a higher level of formaldehyde-induced DPCs, which indicated that *sprtn* gene had an important role in the repair of formaldehyde-induced DPCs. In addition, we investigated whether DPCs affected the repair efficiency of bulky DNA adducts. First, we treated wild-type and *sprtn* knock-down cells with benzo(a)pyrene diol epoxide (BPDE) for 20min, and transferred cells to clean medium for adduct repair. Cells were harvested after 0-hour, 3-hour, 8-hour, and 20-hour repair. We measured the BPDE-DNA adducts by UPLC-MS and we didn't find that the amount of BPDE-DNA adducts had significant difference between wild-type and *sprtn* knock-down cells in each time point. Then, we treated cells [$^{13}CD_2$]-formaldehyde for 2 hours, following with 20 min BPDE treatment. Likewise, cells were transferred to clean medium and harvested after 3-hour, 8-hour, and 20-hour repair. The data indicated that, in presence of formalde-

hyde exposure, *sprtn* knock-down cells have over 400 fold higher BPDE-DNA adducts than wide-type cells in each time point, which was attributed to reduced DNA repair due to increased formaldehyde-induced DPCs. In conclusion, our results demonstrate for the first time that SPRTN plays an important role in the repair of formaldehyde-induced DPCs and formaldehyde-induced DPCs block the repair of BPDE-DNA adducts.

2698 The Role of Genotoxicity in the Carcinogenicity of Acrylamide in the Lungs and Harderian Glands of Mice

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Introduction: Acrylamide (AA) is a contaminant in heated foods and is carcinogenic in multiple organs of rodents. There have been many reports demonstrating AA-induced DNA damages and mutagenicities in *in vivo*. Our previous study showed that exposure to AA at higher dose (400 ppm) for 4 weeks resulted in N7-(2-carbamoyl-2-hydroxyethyl) guanine (N7-GA-Gua) formation and gene mutations in the lungs of *gpt* delta mice. However, in mice treated with AA at a carcinogenic dose (50 ppm), there were no changes in the mutant frequencies (MFs) in the lung in spite of detection of N7-GA-Gua to some extent. We hypothesized that the seemingly incompatible outcome resulted from inadequate duration of the exposure. In the present study, we examined the effects of long-term exposure to AA at the carcinogenic dose on formation of AA-specific DNA adduct and frequency of gene mutations in the carcinogenic target or non-target sites. Method: 6-weeks old B6C3F$_1$ *gpt* delta mice were treated with AA at a dose of 50 ppm in the drinking water for 4, 8 and 16 weeks. After the treatment, lungs and harderian gland as the carcinogenic target sites, and liver as the carcinogenic non-target site, were collected for N7-GA-Gua analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS) and for reporter gene mutation assay. Results: N7-GA-Gua adducts were detected at the almost similar level among the lungs, harderian glands and livers from 4 weeks. The prolonged duration of the exposure did not affect N7-GA-Gua levels at any sites. *gpt* MFs significantly increased in the lungs and the harderian glands at 16 weeks, but not in the livers, and there were no changes in Spi$^-$ MFs at all organs examined at any points of time. Mutation spectrum analysis of *gpt* mutants revealed increases of G:C-T:A transversion and single base deletion in both lungs and harderian glands of mice at 16 weeks. Discussion: AA at the carcinogenic dose significantly increased *gpt* MFs only at the carcinogenic target site, suggesting that mutagenicity of AA might contribute to tumorigenesis in the lungs and the harderian glands. In addition, N7-GA-Gua formation and subsequent AP site formation might be responsible for mutation spectra characterized by increases in G:C-T:A transversion and deletion mutations. However, in addition to the overall data in the livers observed in the present study, the fact that glycidamide (GA), a causative metabolite of N7-GA-Gua, is a rodent liver carcinogen raises a question of whether the genotoxicity starting from N7-GA-Gua formation is involved in AA-induced carcinogenesis. Thus, the possible epigenetic alterations induced by AA may partly contribute to AA tumorigenesis.

2699 Pyraclostrobin Increases HTT CAG Repeats in Cellular Models of Huntington's Disease

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Huntington's disease (HD) is a neurological disorder associated with excessive CAG trinucleotide repeats in the coding region of the Huntingtin (*HTT*) gene. Expansion of greater than 36 CAG repeats elicits pathogenic gene products and a late onset, terminal form of neurodegeneration. The length of CAG and other nucleotide repeat sequences throughout the genome is unstable with a high potential to expand. While HD is primarily inherited, a small proportion of cases arise through sporadic CAG repeat expansions. More CAG repeats are associated with earlier onset; approximately 60% of the variance in the onset in HD symptoms is attributable to the number of CAG repeats a person carries. Environmental exposures may cause CAG repeat expansions that could underlie earlier age of onset of HD as well as sporadic cases of the disease. Yet, we do not know which exposures contribute to repeat instability in trinucleotide repeat disorders such as HD. We previously demonstrated that strobilurin fungicides such as pyraclostrobin cause oxidative stress in mouse neurons and elicit gene expression signatures concordant with human HD. Predicted human pyraclostrobin exposure levels are sufficient to impair human mitochondrial function, and thus, potentially contribute to HD through increasing *HTT* CAG repeat expansion. We demonstrated that exposure to pyraclostrobin caused repeat expansion in a CAG-GFP reporter cell line. Repeat expansion was validated with small pool PCR and molecular fragment analysis. To determine if pyraclostrobin increases CAG repeats in HD-relevant target cells and tissues, we prepared primary cortical cells and fibroblasts from neonatal *Htt* CAG knockin mice. We found no evidence of CAG repeat expansion in mutant cortical cultures exposed to pyraclostrobin under the conditions tested. Mutant fibroblasts showed rare or modest expansion upon chemical treatment. Mutant cortical neurons and fibroblasts showed baseline differences in cell viability and mitochondrial superoxide production relative to wild-type controls. These differences may confound the influence of exogenous mitochondrial stressors on CAG repeat status. Further work is necessary to identify the conditions under which chemical exposure is capable of expanding CAG repeats *in vivo*. Our results re-define the role of environmental chemicals as mutagens underlying rare monogenic diseases and provides an experimental framework to identify chemical threats to trinucleotide repeat stability.

2700 Application of Multiple *In Vitro* Genetic Toxicology Endpoints Enables Comprehensive Characterization of Test Compounds' Mode/Mechanism-of-Action

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The development of several automated *in vitro* and *in vivo* genotoxicity assays in our laboratory presents an opportunity for cross-method comparisons. We identified 7 genotoxicants that had been studied across each of 3 *in vivo* assays - blood and liver micronucleus (MN) and blood-based *Pig-a* gene mutation. The chemicals were: benzo[a]pyrene (BP), cisplatin, cyclophosphamide, hydroxyurea (HU), melphalan, temozolomide, and vinblastine (VIN). These agents were selected for further investigation via *in vitro* MultiFlow to provide data on genotoxic mode of action (MoA) and mechanistic information. This approach used human TK6 cells to examine 73 compounds - including the 7 above - with *a priori* genotoxicity information including *in vitro* MN data. MultiFlow dose response data were converted to concentration-normalized area under the curve values for each chemical to enable unsupervised clustering based on MoA. 22/26 clastogens, 13/13 aneugens and 34/34 nongenotoxic agents were identified correctly. The positive compounds were applied to second tier mechanistic assays to identify direct vs indirect DNA-interacting clastogens or aneugens acting via tubulin disruption or Aurora Kinase inhibition. These analyses confirmed *a priori* expectations of the 7 example agents whereby HU grouped with known non-DNA reactive clastogens, VIN grouped with tubulin destabilizers and the remaining compounds clustered with direct DNA-reactive clastogens. Each group was further characterized based on benchmark dose analyses and potency ranking across the various assays. For example, p53 response ranked VIN as the most potent and BP as the least, this relationship was also observed in both *in vitro* and *in vivo* MN assays. These studies demonstrate the utility of a tiered system of assays where *in vitro* methods provide specific mechanistic information on the type of genotoxic hazard present and thus aid in design of subsequent *in vivo* studies. It bears noting that the majority of compounds studies here are pharmaceuticals with specific activities and do not represent the entirety of potential genotoxicants. Additional chemical classes remain to be studied. This strategy for comprehensive assessment of compounds not only provides detail on genotoxic mechanism, but can conceivably benefit *in vitro* to *in vivo* extrapolation and risk assessment.

2701 Determination of the Genotoxic Potential of E-cigarette Aerosols and Reference Cigarette Smoke in the Mechanism-Based ToxTracker Assay

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The genotoxic assessment of chemicals is a fundamental part of a risk assessment. Studies utilising the *in vitro* micronucleus and Ames assay have shown that *my*blu™ e-cigarette whole aerosol is non-genotoxic under test conditions. In this study we aimed to determine and compare the genotoxic potential of the aerosol/smoke bubbled extracts from the *my*blu™ e-cigarette and 1R6F reference cigarette in the Tox Tracker/ACE assay. ToxTracker is a panel of mammalian stem cell lines that can detect the chemical induction of genotoxicity / cancer pathways through different fluorescent reporters for induction of DNA damage, oxidative stress and protein damage. Smoke/aerosol was generated using an intense smoking regime for 1R6F cigarette (55mL/2s/30s) and the CORESTA Recommended Method N°81 (55mL/3s/30s) for *my*blu™ e-cigarette with 3 different tobacco flavoured liquids (with either

1.6% protonated, non-protonated or 0% nicotine) and unflavoured, based e-liquid without nicotine. Samples were prepared by bubbling through 3 in-line Impingers each containing 10 mL Phosphate Buffered Saline. All samples were tested at a maximum testing concentration of 10%. The differential induction of the green fluorescent protein (GFP) reporters as well as cytotoxicity of the tested compounds was determined using flow cytometry after 24h exposure. In the ToxTracker ACE assay, cell cycle distributions were measured after 4 and 24h. The 1R6F smoke extracts activated both reporters for genotoxicity: Bscl2-GFP, which is activated upon formation of bulky DNA lesions and subsequent DNA replication stress and Rtkn-GFP, which is activated upon induction of DNA double strand breaks, either in absence or presence of S9. Similarly, 1R6F smoke extracts also activated both reporters for oxidative stress, Srxn1-GFP, Blvrb-GFP and the p53 response. Small increases in G2/M phase cells was observed but no increases in aneuploid cells was observed in the ToxTracker ACE assay. None of the aerosols generated with the *my*blu™ e-cigarette were classified as geno- or cytotoxic up to the top testing concentration under the conditions of test. The results obtained highlight the potential utility of this assay for future genotoxicity assessment strategies for tobacco and nicotine-containing products.

PS 2702 Is Endoreduplication an Important Genotoxic Lesion?

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The *in vitro* mammalian cell chromosomal aberration assay is designed to assess structural aberrations and may not be optimal for the assessment of aneuploidy. Since there was no well-validated *in vitro* assay available for the routine screening of aneuploidy, the working group in the first IWGT agreed that many compounds which induced polyploidy also caused aneuploidy *in vitro* and recommended polyploidy (including endoreduplication) should be monitored when observed in the chromosomal aberration assay. Polyploidy induction is used in the chromosomal aberration assay to identify compounds with aneugenic potential as stated in OECD 473 and ICH S2(R1). However, there is a lack of scientific evidence showing that polyploidy is a reliable surrogate for aneuploidy and there are few publications which have included data on spontaneous or induced polyploidy. To better understand the correlation, we retrospectively evaluated the historical data generated in WuXi AppTec for CHO-WBL chromosomal aberration assay. In our laboratory, we recorded the polyploidy and endoreduplication separately and the induced polyploidy or endoreduplication beyond the historical negative control data range was considered positive. In the past two years, 79 CHO-WBL chromosomal aberration studies were conducted. The overall frequencies of positive for polyploidy and endoreduplication were 5% (4/79) and 48% (38/79), respectively. In our studies on pharmaceutical compounds endoreduplication was the lesion most often found, being induced by 27% in short treatment with S9, 39% in short treatment without S9 and 9% of the compounds tested in long treatment without S9. Most of those findings occurred close to the 50% cytotoxicity limit, nearly 86% of positive points are with more than 30% cytotoxicity. Of 38 candidates positive in endoreduplication, two candidates have no *in vivo* data, 4 compounds are positive comprising two with known mechanisms (CDK inhibitor and red cell proliferating agent) and other two kinase inhibitors with no known mechanism, but almost 89% (32/36) of candidates are negative in *in vivo* micronucleus assay, which indicating endoreduplication is clearly over-predicting the frequency of truly aneugenic compounds. Our data showed endoreduplication is seen quite often in *in vitro* CHO-WBL chromosomal aberration assay and not usually indicative of genotoxic (aneugenic) potential as the positive responses are usually not confirmed in *in vivo* micronucleus assay.

PS 2703 Application of High-Throughput CometChip Technology and Quantitative Approaches in Primary Human Hepatocytes

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Primary human hepatocytes (PHHs) are considered as the "gold standard" for evaluating hepatic metabolism and toxicity of xenobiotics. Previously, we evaluated the *in vitro* genotoxicity of a set of genotoxic carcinogens that have different genotoxicity and carcinogenicity modes of action in metabolically competent HepaRG and HepG2 cells. In this study, we used cryopreserved PHHs derived from three individual donors to evaluate the genotoxicity of eleven genotoxic carcinogens requiring metabolic activation (indirect-acting) or not (direct-acting) and five genotoxic non-carcinogens. DNA damage effects of the 16 compounds were determined over a wide range of concentrations using the high-throughput CometChip technology and the resulting dose-responses were quantified using benchmark dose (BMD) modeling. Following a 24-h treatment, nine out of eleven genotoxic carcinogens showed positive responses in PHHs, while negative responses were found in two known aneugens. PHHs gave an overall sensitivity of 100% and 71% for detecting DNA damage of six indirect-acting and five direct-acting genotoxic carcinogens, respectively, which were much higher than did HepG2 and HepaRG cells (i.e., 50-75% and 57% for detecting indirect-acting and direct-acting carcinogens, respectively). In addition, PHHs showed a specificity of 100% for detecting the five genotoxic non-carcinogens. Both BMD_{1SD} and BMD_{10} values were calculated using BMD analysis for the DNA damage dose-responses. HepaRG cells and PHHs showed comparable BMD_{1SD} values among the three types of hepatocytes, whereas similar BMD_{10} values were observed between HepaRG and HepG2 cells. These results suggest that PHHs can be adapted to 96-well CometChip platform for facilitating high-throughput genotoxicity evaluation and that quantitative approaches can be useful in support of developing *in vitro* cell models for genotoxicity testing.

PS 2704 Performance of HepG2 and Metabolically Competent HepaRG Cells in High-Throughput Micronucleus Assay for *In Vitro* Genotoxicity Assessment

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Micronucleus (MN) test is a core assay for *in vitro* genotoxicity testing. The traditional MN assay is usually conducted in cells lacking metabolic competency or relying on exogenous metabolic activation systems, which is an important drawback of the current *in vitro* systems for genotoxicity assessment. Our previous study demonstrated that compared to HepG2 cells, HepaRG cells expressed a much higher level of CYP450 enzyme activities and detected a greater portion of genotoxic carcinogens requiring metabolic activation (indirect carcinogens) in the high-throughput high-content (HTHC) *in vitro* Comet assay. In this study, we evaluated the performance of HepG2 and HepaRG in HTHC *in vitro* MN assay for genotoxicity evaluation. Both cell lines were treated with wide-ranging concentrations of twelve genotoxic (seven direct and five indirect) carcinogens for 24 h and cultured for additional hours to go through 1.5-2 cell cycles. The results demonstrated that four out of twelve carcinogens showed different responses in these two cell lines. Specifically, three chemicals that require metabolic activation (i.e. 7,12-dimethylbenz[*a*]anthracene, cyclophosphamide, and *N*-nitrosidimethylamine) induced significant increases in MN formation in HepaRG cells when compared to the vehicle control, while no significant MN production was observed in HepG2 cells. Cadmium chloride was positive in HepG2 cells, but negative in HepaRG cells. HepaRG cells showed a much higher sensitivity (80%) than HepG2 cells (20%) in detecting five indirect carcinogens; whereas HepG2 cells showed a higher sensitivity (100%) than HepaRG cells (86%) in measuring seven direct carcinogens. 2,4-Diaminotoluene was negative in both cell lines. These results suggest that HTHC flow cytometry-based MN assay can be adapted to HepaRG cells for genotoxicity assessment, and HepaRG cells are superior than HepG2 cells in detecting carcinogens that require metabolic activation.

PS 2705 Genotoxicity Assessment of DMN and DEN in Cultured Human Peripheral Blood Lymphocytes

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Dimethyl or diethyl nitrosamine (DMN and DEN) are known liver carcinogen in rats. Various *in vitro* tests have been successfully detected mutagenic potential of these nitrosamines. The *in vitro* tests that has been used mainly involves bacterial system and immortalized mammalian cells originated from rodents. Immortalized mammalian cells are not a true representative of the normal cells of human since some of the important genes are either absent of mutated in cell lines. We investigated the genotoxicity potential of DMN and DEN in cultured human peripheral blood lymphocytes (HPBL) using cytochalasin blocked micronucleus assay (OECD 487). Aroclor-1254-induced rat-liver S9 was used as metabolic system (5% v/v). Current test OECD 487 testing guideline recommend maximum concentration that should be tested is 10 mM or 2 mg/mL, whichever is less. We performed series of experiment using different donors and evaluated micronucleus frequency. DMN and DEN did not induce statistically significant micronucleus frequency up to 10 mM final concentration in culture (DMN =740 µg/mL; DEN = 1021 µg/mL). However, when concentrations were increased to 6000 µg/mL and above, which is equivalent to 8x (DMN) and 6x (DEN), respectively of OECD 487 recommended maximum concentration (10 mM), we observed significant induction of micronucleus without much cytotoxicity. Our results indicated that

DMN, DEN and therefore related chemicals with low molecular weight will be missed during hazard assessment when tested in HPBL test system according to OECD 487 recommended concentration.

2706 DNA Damages Induced by *Ortho-*, *Para-*, and *Meta*-Chloroaniline Related to the Occurrence of Occupational Bladder Cancer

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More than ten cases of occupational bladder cancer were identified in a chemical factory in Japan, and *ortho*-toluidine, a group I carcinogen as classified by the International Agency for Research on Cancer (IARC), was thought to be the main agent for the disease. However, some other aromatic amines including *ortho*-chloroaniline (OCA) were also used there. OCA has not been assessed for its carcinogenesis by IARC, and its possible role in the occurrence of the occupational bladder cancer is not clear. In this study, we investigated the genotoxic effect of OCA and compared this with its structural isomers, *para*- and *meta*-chloroaniline (PCA and MCA, respectively) as well as aniline, the simplest aromatic amine and group III carcinogen in IARC classification. Human urothelial (1T1) and hepatocyte (WRL-68 or HepG2) cells were treated with OCA, PCA, MCA or aniline at different concentrations for 2-24 hr, and phosphorylated histone H2AX (γ-H2AX), a marker of DNA double strand breaks, and damaged DNA were detected by western blot and pulse field gel electrophoresis, respectively. Results: OCA showed significant γ-H2AX induction both in urothelial and hepatocyte cell lines, and this effect was even stronger than *ortho*-toluidine. PCA and MCA also showed γ-H2AX induction effect. The patterns of induction were different among the three chloroaniline isomers: the induction of γ-H2AX by OCA reached peak at 4 hr and almost disappeared at 24 hr after addition, while the induction by PCA and MCA was becoming strong up to 16-24 hr. The DNA double strand breaks induced by the three isomers of chloroaniline was confirmed on gel electrophoresis. It is speculated that the chloro group position may affect the DNA damaging and/or repairing ability through the metabolic activation. Our results suggested that chloroaniline is a strong DNA damaging agent, and it could play a critical role in the occurrence of occupational bladder cancer if the workers are exposed to the chemical. Further assessment on the carcinogenesis of chloroaniline is necessary for the protection of workers' health.

2707 High-Throughput *In Vitro* Micronucleus Assay in Human Lymphoblastoid Cells

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Micronuclei are chromosome fragments or whole chromosomes that are not incorporated into one of the daughter nuclei during cell division. The *in vitro* micronucleus assay evaluates genotoxic damage by detecting the formation of micronuclei in the cytoplasm of interphase cells. It is a well-established and widely accepted genotoxicity test in preclinical safety assessments to analyze the potential risk for genetic damage in cultured cell lines and primary cells. Traditionally, the *in vitro* micronucleus assay requires a labor-intensive and time-consuming microscopy-based analysis of the treated samples which is low throughput. Here we report a 96-well plate version of the *in vitro* micronucleus assay in which automated scoring of micronuclei and cytotoxicity is achieved by cell-staining approaches with flow cytometer analysis. The p53 proficient and karyotypically stable human B lymphoblastoid cell line, TK6, was used in the assay to reduce the high rate of false positive results that is seen in p53-mutated cell lines and to avoid the donor variability that is seen with human peripheral blood lymphocytes. Seven reference compounds, with positive and negative genotoxic effects, each were tested at 16 dose levels to evaluate the potential to induce micronuclei in TK6 cells in both the absence and presence of an exogenous metabolic activation system. Cytotoxicity and fold-increase in apoptosis/necrosis relative to the vehicle control were also measured. The assay demonstrated a good correlation between the MNT results obtained by the high-throughput assay and the genotoxicity profile of these compounds. The results confirmed that the method may be applied for genotoxicity screening in the pharmaceutical and chemical industry.

2708 Humidity Influence on Slide Preparation in Chromosomal Aberration Assay

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The *in vitro* mammalian cell chromosomal aberration assay is used to assess potential genotoxic hazard of test articles and has been used quite extensively in the pharmaceutical industry. In this assay, establishing a method for slide preparation and ensuing quality is particularly important and worth spending on extra effort because it will improve the reliability of results and greatly reduce slide scoring time and technician fatigue in subsequent routine experiments. It is well known that there are differences in chromosome spreading with humidity, however, there is very limited article to elucidate the humidity influence and the optimized humidity for slide preparation. In the past two years, we've been monitoring the humidity in the slide-making area. In this abstract, the seasonal variations are evaluated and an optimized humidity range is proposed. The objective of successful slide preparation is to ensure that the metaphase spreads are kept intact, but the chromosomes are separate and there is as little cytoplasm as possible. In our laboratory, we achieve this by a number of cunning ruses, such as using wet slides, using humidifiers and dehumidifiers to adjust the humidity, by dropping cells from a height to improve spreading or by warming the slides on slide warmer. Before slide preparation, we record the humidity first and then make the slides to check whether the quality meet our criteria, if not, the humidity will be adjusted until the slide can be made successfully. With the air-conditioning, the temperature is stable and around 25°C, but seasonal humidity varies obviously from 19% to 82%. From Oct. to Mar., the humidity are generally below 60% and excellent preparations can be made without adjusting humidity, occasionally the humidifiers will be used to increase the humidity when it is below 30%. From Apr. to Sep., the humidity are generally above 60% and the slides can't be made perfectly without adjusting the humidity, the dehumidifiers will be used to decrease the humidity, sometimes slides will be placed on the vent of dehumidifiers to improve the spreading. Based on about 80 times of slide preparation, the humidity range of 40% to 50% was proposed as the optimized range for slide-making in our laboratory. Our data showed high seasonal humidity variations impacted slide-making and excellent preparations can be made with humidity adjusting. We therefore suggest to monitor and record the humidity in the slide-making area for reference purposes.

2709 Determining Genotoxic Potential and Mode-of-Action in a Single Mammalian Cell Assay

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Numerous efforts within the genetic toxicology field aim to move beyond measuring only apical endpoints to assess genotoxic potential. More emphasis is being placed on understanding key molecular events (KEs) in adverse outcome pathways (AOPs) that elicit genotoxicity. Presently, there are assemblies of AOPs (some draft) for DNA reactive and non-reactive modes of action. These biological networks can be used to focus testing strategies on identifying upstream KEs responsible for an observed apical endpoint. Thus, a comprehensive all-in-one mammalian cell assay that can screen for genotoxic potential and assist in identifying relevant AOPs would be of value. Therefore, we implemented flow cytometric analysis of micronuclei (MN) in CHO cells since such scoring facilitates rapid acquisition of induced MN and, uniquely, the CHO test system can discriminate aneuploid daughter cell nuclei that survived non-disjunction (a KE in aneugenic AOPs). Several clastogens (benzo[a]pyrene, cyclophosphamide, etoposide, methyl methanesulfonate, mitomycin C, and quinoline) were tested in a 24-well format with and without metabolic activation (S9) to establish laboratory proficiency prior to challenging the assay with three reference aneugens (carbendazim, colchicine, and vinblastine) and amsacrine (AMSA), a DNA intercalating agent that inhibits topoisomerase II (topo-II). Cells were processed as per the appropriate MicroFlow® kit (Litron Laboratories; Rochester, NY), and up to 10,000 nuclei were analyzed on a BD FACSCanto II. All chemicals evaluated were genotoxic, with significant increases in %MN observed (as compared to the vehicle control; $z' > 0.6$). However, only the three aneugens and AMSA induced significant (>10%) hypodiploidy, a measurement of non-disjunction which is a KE in aneugenic AOPs. Except for AMSA, our results confirm published findings in mammalian cell systems. AMSA and other topo-II inhibitors have shown aneugenic activity *in vivo*, including in germ cells, which is something that was not observed for etoposide during assay proficiency testing. Hence, follow up work will include closer examination of etoposide's dose-response relationship (*e.g.*, testing up to suitable cytotoxicity limits, modifying concentration spacing, etc.) and evaluation of other Topo-II poisons in this assay.

2710 Development of TK6-Derived Cells Expressing Human Cytochrome P450s for Genotoxicity Testing

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Metabolism plays a key role in chemical genotoxicity; however, most of mammalian cells used for *in vitro* genotoxicity testing are devoid of metabolizing enzymes. We recently developed a battery of TK6-derived cell lines that individually overexpressed one of eight cytochrome P450s (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C9, 2C19, and 3A4) using a lentiviral expression system. The increased expression and metabolic function of the individual CYP in each established cell line were confirmed using real-time PCR, Western blotting, and mass spectrometry analysis, while their parental TK6 cells and empty vector (EV) transduced cells had negligible CYP levels. Subsequently, we validated these cell lines using two prototypical polyaromatic hydrocarbon mutagens, 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (B[a]P), that require metabolic activation to exert their genotoxicity. DMBA induced-cytotoxicity, phosphorylation of histone H2A.X, and micronucleus formation were significantly increased in TK6 cells with CYP1A1, 1B1, 2B6, and 2C19 overexpression as compared to EV controls. B[a]P significantly increased cytotoxicity, DNA damage, and chromosomal damage in TK6 cells overexpressing CYP1A1 and 1B1 when compared to EV controls. B[a]P induced-micronucleus formation was also found in TK6 cells overexpressing CYP1A2. These results suggest that our CYP-expressing TK6 cell system is capable of detecting the genotoxicity for compounds that require metabolic activation.

2711 Quantitative Analysis of Aristolochic Acid-Induced Genotoxicity in Rats

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Aristolochic acid (AA) is a group of structurally-related nitrophenanthrene carboxylic acids found in many plants that are widely used by many cultures as traditional herbal medicines. AA is the causative agent for Chinese herbs nephropathy, a term replaced later by aristolochic acid nephropathy (AAN). Evidence indicates that AA is nephrotoxic, genotoxic, and carcinogenic in humans; it induces tumors in forestomach, kidney, renal pelvis, urinary bladder, and lung of rats and mice. Therefore, plants containing AA have been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer. We have conducted a series of genotoxicity and toxicogenomic studies in the rats exposed to AA of 0.1-10 mg/kg for 12 weeks, and the results demonstrated that AA treatments induced DNA adducts and *mutations* in the kidney, liver, and spleen of rats, as well as significant alteration of gene expression in both its target and non-target tissues. We also applied benchmark dose (BMD) modeling to the 3-month AA-induced genotoxicity data. The $BMDL_{10}$s (the lower 95% confidence interval of the BMD_{10} that is a 10% increase over the background level) for inducing *cII* mutations in the kidney, liver, and spleen were 8, 41, and 286 µg/kg/day, respectively. For the H-*Ras* mutations, the $BMDL_{10}$ for kidney (7 µg/kg/day) was about 4-fold lower than that of liver (28 µg/kg/day). The two $BMDL_{10}$ calculated from the dose-response curves of *cII* mutant frequency and H-*Ras* mutation in the kidney were very close, and the obtained $BMDL_{10}$ for AA-induced *mutations* in the kidney of rats was about 7-8 µg/kg body weight per day. With modifying factors (uncertainty or safety factors), the permitted daily exposure (PDE) defined as a pharmaceutically acceptable intake can also be calculated. The more ingestion or inadvertent exposure to AA-containing herbal products, the higher the risk of human diseases, including cancer.

2712 Quantitative Measurement of DNA Adducts in Human Saliva by Nano-Liquid Chromatography-Tandem Mass Spectrometry

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There is a critical need to develop a robust method to accurately quantify DNA adducts in the easily accessible specimen which reflects the exposures and responses induced by endogenous exposome and exogenous stimuli. To this end, we developed a high sensitive platform to simultaneously quantify seven kinds of DNA adducts including N_6-Me-dA, N_6-Et-dA, O_6-Me-dG, N_2-Me-dG, N_2-Et-dG, 1,N_2-Etheno-dG, and 1,N_2-Propano-dG using synthetic stable isotope labeled internal standards. This targeted LC-PRM (parallel reaction monitoring) assay analysis was conducted using an UltiMate 3000 RSLCnano system coupled to a Q Exactive HF mass spectrometry. Those seven DNA adducts of targets can be well-separated based on their retention time and accurate mass (<5 ppm mass accuracy) of monitoring product ions in one single run. The detection sensitivity of our optimized assay is able to reach attomole (3.9 attomole for 1,N2-Etheno-dG and 1,N2-Propano-dG; and 1.95 attomole for N2-Me-dG and O6-Me-dG) and sub-attomole levels (0.97 attomole for N2-Et-dG and 0.488 attomole for N6-Me-dA and N6-Et-dA). The calibration curve for each DNA adduct is individually established by plotting the peak area ratios of solutions containing a fixed concentration of isotope labeled internal standard and increasing concentrations of analytical standard without labeling. Moreover, the LC-PRM assay is thoroughly validated by examining the intra- and inter-day accuracy and precision, resulting both less than 15% relative standard deviation (RSD) indicating the robustness of our quantification platform. Next, we applied our assay to detect and quantify of those DNA adduct targets from human saliva samples. In brief, the saliva DNA was extracted and spiked with isotope labeled internal standards prior to digestion to release the nucleosides. The target DNA adducts were purified by off-line reversed-phase fractionation, and thereafter LC-PRM assay analysis. Using our optimized sample preparation and platform, target DNA adducts in human saliva can be successfully quantified. Overall, our study provides a significant improvement and facilitates further applications for quantification of DNA adducts in the easily collected saliva, providing a comprehensive evaluation of human exposome and serving a useful tool to quantify DNA adduct biomarkers for risk assessment of chemicals.

2713 Validation of Duplex Sequencing for *In Vivo* Mutation Detection: Interspecies Comparison

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Transgenic rodent mutation assays are the gold-standard for measuring *in vivo* mutagenicity. The Big Blue® assays (OECD Test Guideline 488) use transgenic rodents to measure mutations by recovering an integrated shuttle vector through a labor-intensive plaque-based method but provide no genomic information. Duplex Sequencing™ (DS) is a Next Generation Sequencing error correction method that reduces the sequencing error rate by 100,000-fold. This permits measurement of mutant spectra. and for the first time, of mutant frequencies less than 1×10^{-7} for any species, tissue, sex, or DNA segment. Here, we compared DS to the plaque-based assay in BB mice and then extend our analysis into endogenous genes in BB mice and then BB rats. A 15 KB probe set was used in mice focusing on introns and exons of 5 genes. In rats a probe set of 50 KB was selected independent of biological function from approximately equal sized segments from all autosomal chromosomes. Similar low background mutant frequencies and mutant spectra were observed in control mice and rats. Both ethyl nitrosourea (ENU) and benzo(a)pyrene significantly elevated mutant frequency in liver in both species. In mice, the *cII* transgene was included in the DS probe set. In this bridging study the *cII* plaque and DS methods yielded similar fold increases in mutant frequency and shifts in mutant spectra showing equivalence of the two analytical methods to detect mutagenesis. In rats, without *cII* included in the DS probe set, similar patterns of response were seen in the mutant frequency in *cII* plaque and DS in endogenous loci. In both species, unsupervised cluster analysis of simple mutant spectra and trinucleotide spectra showed that each treatment condition produced similar patterns in mice and rats but that there were unique signatures associated with each treatment condition. The data show that DS is equivalent, and in many ways better, at detecting mutations in the *cII* gene. DS is robust in detecting changes in mutant frequency and mutant spectra in endogenous DNA in two rodent species. In summary, our results show that DS is an acceptable method for measurement of *in vivo* mutagenesis and is promising as a new nonclinical biomarker of human cancer risk. DS mutation analysis could be added into any repeat dose study using any wild-type species.

2714 Evaluation of Micronucleus Induction in Erythrocytes of Chicken Embryo-Fetuses Using Flow Cytometry and Laser Scanning Cytometry Approach

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The micronucleus assay is a widely used test for detection of mutagenic potential of xenobiotics. The use of techniques such as flow cytometry for detection of micronuclei formation significantly enhances the assay, allowing for high throughput analysis and elimination of several shortcomings of conventional microscopy. Thus, in the current study, flow cytometry and laser scanning cytometry (LSC) were used to evaluate micronuclei formation in erythrocytes of chicken embryo-fetuses in the novel animal-alternative model, Chicken Egg Model (CEM), after exposure to an establish mutagen methyl

methanesulfonate (MMS). For this purpose, white leghorn chicken eggs of undetermined sex were injected with either 20% aqueous solution of Kolliphor® HS15 oil (20% HS-15), which served as a vehicle, or 0.83 mg/egg of MMS on day 9 of incubation followed by collection of blood samples for micronuclei evaluation 24 hours after the injection time on day 10. For flow cytometry detection we utilized the *in vitro* MicroFlow Kit (Litron Laboratories) protocol with few variations, including use of DAPI stain to visualize erythrocyte nuclear DNA. We observed significant increases in the micronuclei formation using blood collected from MMS treated eggs (1.74%) when compared to blood collected from control group (0.70%). In order to establish an optimal detection method, we conducted laser scanning cytometry (LSC) as well as microscopy and both the techniques confirmed the results observed in flow cytometry. Micronuclei formation using LSC in MMS dosed eggs (8.57%) was significantly increased when compared to the control group (3.8%). In addition to blood, livers were also collected for comet assay. MMS produced an increase in DNA strand breaks in the liver (17.4% of DNA in the tail compared to 4.56% in control group). Thus, the results of this study confirms that high throughput techniques, such as flow cytometry and LSC are reliable methods for the detection of micronuclei induction in CEM.

2715 Evaluation of Crop Protection Products for Genotoxicity Potential

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Assessment of genotoxic potential is an important component of the safety evaluation process of crop protection (CP) active ingredients (a.i.) and their formulated products (FP). Each a.i. is thoroughly tested for genotoxic potential using bacterial and mammalian tests, *in vitro* and *in vivo*. Upon any positive or equivocal results, a.i.'s is further evaluated in higher-tier genotoxicity tests. Active ingredients are formulated either singly or in mixtures with other a.i's in diverse formulation types. In addition to a.i.(s), CP formulations contain multiple co-formulants, including solvents, surfactants, etc. There is increasing interest to investigate the effect of co-formulants on the genotoxic potential of the final FP. The purpose of this retrospective evaluation was to examine whether genotoxicity results of FPs are consistent with corresponding a.i.(s) and further to evaluate whether co-formulants enhanced the genotoxic hazard of the final FP. In this study, genotoxicity (Ames and *in vivo* micronucleus tests) data for 156 commercial CP formulations and their corresponding a.i.(s) (n=86) and co-formulants were compiled and assessed. The genotoxicity data for a.i.'s was obtained from GLP studies (followed OECD TG) and the data for co-formulants was compiled from ECHA dossiers and safety data sheets. The FPs consisted of 1-3 a.i's and up to 13 co-formulants and comprised of both solids and liquids representing a total of 16- formulation types. The a.i. concentrations in FP ranged from 2 to 75% and co-formulant concentrations up to 85%. In many cases, each a.i was formulated in various forms (salts, acid, amine etc) and in multiple FP (up to 19). Evaluation of the data for a.i.'s and co-formulants suggested lack of genotoxic potential at tested concentrations. The CP formulations data indicated that genotoxicity potential did not change compared to corresponding a.i.'s irrespective of concentrations and number of a.i.'s or co-formulants in each formulation and formulation type. Furthermore, all evaluated FPs were negative for genotoxicity. In conclusion, these results suggested that the combination of a.i's and co-formulants did not increase genotoxicity in a representative group of diverse type of formulations. These data support that additional tests for formulations are not warranted to confirm genotoxicity potential when a.i's are extensively evaluated and all selected co-formulants are non-genotoxic.

2716 Impact of Glutathione (GSH) on the Mutagenicity and Cytotoxicity of Methyl- and Ethyl Acrylate in the Mouse Lymphoma Assay

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Ethyl Acrylate (EA) and Methyl Acrylate (MA) are monomer building blocks in the production of polymers and copolymers. The genotoxicity and mutagenicity data sets support a cytotoxic, non-genotoxic mechanisms for these acrylates. EA did not induce mutations in a Transgenic mouse assay *in vivo* (Ellis-Hutchings et al, 2018), whereas treatment of LY5178 mouse lymphoma cells *in vitro* with MA or EA in the thymidine kinase gene mutation assay led to a significant increase in the mutation frequency of the treated cells (Moore et al, 1988). An increased mutation frequency was only observed at cytotoxic concentrations. Considering the high binding affinity of the compounds to the intracellular antioxidant Glutathione (GSH), and known propensity of EA and MA to acutely deplete GSH *in vivo*, we postulated that the observed effects *in vitro* might derive from a depletion of the GSH. In the present study the impact of exogenously supplemented GSH on the mutagenicity and cytotoxicity of MA and EA was analyzed. Pulse treatment of LY5178 mouse lymphoma cells with either MA or EA (10-35 µg/mL) induced concentration dependent cytotoxic effects (relative total growth of 14.1 and 10.2% after treatment with 30 and 35 µg/mL of MA and EA, respectively). Supplementation of the treated cultures with 100 µM GSH abrogated both the observed cytotoxicity as well as the mutagenicity. The quantification of the GSH levels in the cell lysates after the treatment period showed a concentration related decrease in the intracellular GSH levels (down to 18.2 and 13.4% of the corresponding controls for MA and EA, respectively). The present data demonstrate that MA or EA decreases intracellular GSH levels in LY5178 mouse lymphoma cells *in vitro*. The cytotoxic and mutagenic effect was attenuated in a concentration-dependent manner by GSH supplementation. These observations suggest that the previously observed mouse lymphoma mutagenicity of MA and EA was likely due to a depletion of GSH *in vitro* and may explain the differences in the outcome of *in vitro* versus *in vivo* tests.

2717 Effects of Amlexanox and Other Compounds on DNA Repair in Xeroderma Pigmentosum Group C Cells

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Xeroderma pigmentosum (XP) is a rare autosomal recessive DNA repair disorder caused by mutations in 8 genes, XP-A to -G, and Variant with severe sun sensitivity and10,000-fold increased risk of skin cancer. XP-C is the most common type in the US About 12% of XP-C patients have premature termination codon (PTC) mutations that can lead to XPC protein elongation arrest and *XPC* mRNA template degradation by nonsense mediated decay. Previous studies in our laboratory have shown that levels of expression of *XPC* mRNA and XPC protein in fibroblasts derived from XP-C patients with PTC mutations were enhanced by aminoglycosides. Amlexanox, which is an anti-inflammatory, antiallergic and immunomodulator, was reported to increase readthrough in human cells with PTC associated diseases. Amlexanox is a topical paste indicated to treat several inflammatory conditions. Exposure to amlexanox will enhance the expression of *XPC* mRNA in fibroblasts and lymphoblasts derived from XP-C patients with different PTC mutations and different baseline levels of XPC mRNA. Methods: Parallel pairs of fibroblasts and lymphoblasts derived from two unrelated patients along with 2 normal control cells were exposed to amlexanox and/or our previously tested aminoglycosides. *XPC* mRNA levels were measured using standardized qRT-PCR and the results were compared to normal unexposed cells of each type. Levels of *XPC* mRNA increased in XPC fibroblasts exposed to both G418 and amlexanox to a different extent in different cells. Levels of *XPC* mRNA expression changed with amlexanox in parallel pairs of fibroblasts and lymphoblasts in a similar fashion in both patients. Due to the availability of amlexanox and the other tested drugs in our study commercially as topical dosage forms, they could be used as potential therapeutic agents for increasing functional *XPC* protein with less associated systemic toxicity and thereby decrease XP-C associated skin cancer risk. This preclinical test is a step forward towards precision medicine in which it would help with patients selection and determining which drug combination would be optimal to increase XPC function for each patient.

2717a Acetaldehyde-Induced DNA-Protein Crosslinking in Human Bronchial Epithelial BEAS-2B Cells and Hepatic Stellate Cells

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Acetaldehyde is a common pollutant in air, and also produced by human alcohol metabolism. Acetaldehyde not only causes decreased cell activity, but also induces DNA damage such as DNA-protein crosslinking (DPC) in human cells. DPC blocks the normal transcription and replication of DNA, therefore it can be used as a molecular marker for potential mutations in the body. In the current study, we investigated DNA-protein crosslinking rate both in BEAS-2B cells and hepatic stellate cells (HHSteCs). BEAS-2B cells and HHSteCs were exposed to 4, 8, 10, 12 and 16 mM acetaldehyde for 24h after normally cultured for 24h. DNA-protein crosslinking was estimated by KCl-SDS assay. And DNA was detected by dyed with SYBR Gold nucleic acid stain. The results showed that DPC ratios were significant increased both in BEAS-2B cells and HHSteCs when treated with acetaldehyde. The experiment also indicated a dose-dependent effect. In addition, DPC ratio in HHSteCs was slightly higher than in

BEAS-2B cells, which means HHSteCs might more sensitive than BEAS-2B cells while exposed to acetaldehyde. As an environmental chemical, acetaldehyde induced DNA breakage both in lung cells and liver cells.

PS 2718 Glyoxal-Derived Advanced Glycation End Products Induce Apoptosis in Hepatocytes

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Final products of the non-enzymatic reaction between amino groups of protein, lipids, nucleic acids and reducing sugars are called advanced glycation end-products (AGEs). It has been known that AGEs interact with RAGE, the receptor for AGEs located on the plasma membrane and induce inflammation, and the generation of reactive oxygen species (ROS), triggering oxidative stress. This disrupts the intracellular signaling and gene expression, leading to apoptosis. Apoptosis is highly programmed cell death that is vital for the maintenance of homeostasis in multicellular organisms. Excessive apoptosis on the other hand, leads to acute injuries including fulminant hepatitis and chronic diseases such as viral hepatitis and alcoholic liver disease. According to several studies, AGEs may contribute to hepatic fibrosis and chronic liver disease. Still, the relationship between exposure to AGEs and hepatic apoptosis remains unclear. In this research, HepG2 cells were used to investigate hepatic apoptosis by glyoxal-derived AGEs (glyoxal-AGEs) MTT assay was carried out to assess the cytotoxicity of glyoxal-AGEs. To detect the oxidative stress induced by glyoxal-AGEs, ROS production was measured. Expression level of apoptotic factors such as Bax, Bcl-2, caspase 3, caspase 9 and p53 at mRNA level and protein level were measured using RT-qPCR and Western blot analysis. As HepG2 cells were exposed to glyoxal-AGEs, the production of ROS increased. Additionally, the mRNA level of pro-apoptotic markers such as Bax, p53, caspase 3, and caspase 9 increased while the level of anti-apoptotic maker, Bcl-2 decreased, reducing the Bcl-2/Bax ratio. Western blot analysis also showed that the protein level of same pro-apoptotic markers increased as anti-apoptotic marker Bcl-2 decreased. These results suggest that glyoxal-AGEs may induce ROS-dependent apoptosis in hepatocytes.

PS 2719 Methyl Glyoxal-Derived Advanced Glycation End Products Induced Kidney Cell Apoptosis, Leading to the Endoplasmic Reticulum Stress and Mitochondria Dysfunction through MAPK/JNK Pathway

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Diabetic nephrosis, which occurs in proximal renal epithelial cells, is one of the main complications of diabetic patients around the world. If diabetic necrosis occurs, the function of the endoplasmic reticulum (ER) and mitochondria in the kidney cell will deteriorate, interrupting protein synthesis and reducing ATP synthesis, causing inflammation, fibrosis, and cell death. Recent studies have shown that advanced glycation end products (AGEs) are substances that play an important role in inducing diabetes, Among the AGEs from various sugars, especially, those derived from methylglyoxal have recently been found to be a major cause of diabetes. and interact with AGE for receptors (RAGE) located on the cell surface to control multiple signaling systems with mitochondrial dysfunction. When ER stress signal occurs, ER stress leads to activation of c-Jun N-terminal kinase (JNK) and induction of C/EBP homogenous protein (CHOP). Both JNK and CHOP increase the apoptotic effect of Bax and remove the anti-apoptotic effect of Bcl-2 to express in kidney cells. In this study, methyl glyoxal-derived AGE (200 μg/mL) was treated to human proximal epithelial kidney cells (HK-2), and cell viability was measured. We confirmed that methyl glyoxal-derived AGE combined with RAGE, caused ER stress and affected the membrane potential of the mitochondria as the CHOP is controlled through the phosphorylation of JNK. This confirmed that the renal cell function was impaired by an imbalance in the mitochondrial membrane and interaction with Bax/Bcl-2 that affected cell apoptosis due to inhibiting ATP synthesis. This tendency was demonstrated in animal experiments by oral administration of 800 mg AGEs /kg.bw to C57/BL6 mouse for 21 d to demonstrate the expression of ER stress and cell apoptosis biomarkers Bax/Bcl-2 by methyl glyoxal-derived AGEs. Through *in vitro* and *in vivo* test, we are going to provide a new perspective on the diabetic kidney disease mechanism induced by methyl glyoxal-derived AGE and identify the signal pathway system leading to kidney cell dysfunction through the mitochondria membrane imbalance and ER stress.

PS 2721 Moxidectin, a Novel Therapeutic Option for Pediatric Medulloblastoma

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Pediatric brain tumor is one of the most malignant solid tumors in children and have profound impact on the morbidity and mortality in these patients. Statistically, brain tumors are one of the leading cause of cancer-related deaths in patients from 0-19 years of age. Medulloblastoma (MB) is one of the most common pediatric brain tumors occurring in children. Sonic hedgehog (Shh) activated subgroup of MB is considered to be highly aggressive and metastatic in nature. Shh-MB is characterized by mutations in PTCH1, SMO and SuFu along with amplified activation of Gli1, a major transcription factor of this signaling pathway. In the current study, we have evaluated the anti-cancer effects of an anthelminthic drug 'moxidectin'. Several MB cell lines such as Daoy, UW426, UW228, ONS76, and PFSK1 were treated with moxidectin in a concentration and time dependent manner. Our results demonstrated that moxidectin treatment resulted in significantly reduced proliferation of MB cells. The IC_{50} of moxidectin in all the MB cell lines ranged 10-17μM after 24, 48 and 72 hours of treatment. Moreover, moxidectin was able to induce 3-4 fold increase of apoptosis in all the MB cell lines as evaluated by AnnexinV-FITC/PI assay, and increased cleavage of caspase 3 and PARP. Western blotting analysis demonstrated that moxidectin treatment significantly reduced the expression of Shh and Gli1 and their downstream effector molecules such as Pax-6, Oct-4, Sox-2 and Nanog. Efficacy of moxidectin was evaluated in an *in vivo* tumor model by subcutaneously injecting human Daoy MB cells in the right and left flank of the mice. Our results demonstrated that 5mg/kg and 10 mg/kg moxidectin by oral administration everyday suppressed the growth of Daoy tumors by 70% and 90% respectively. Conclusively, our results indicate that moxidectin effectively reduces the growth of MB tumors by inhibiting Shh signaling. Most importantly, moxidectin is FDA approved drug and is already in clinical use for the treatment of river blindness in humans with an established safety record, therefore any positive findings from our studies will prompt further clinical investigation into repositioning moxidectin for the treatment of MB patients.

PS 2722 Pancreatic Tumor Growth Suppression through Induction of Autophagy by a Novel Anti-Parkinson's Drug, Pimavanserin

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Pancreatic cancer patients have limited treatment options in spite of several advanced treatment strategies. Pancreatic tumors exhibit high basal autophagy compared to other cancers. Several studies including from our lab reported that enhanced autophagy can lead to apoptosis in cancer cells. In this study, we have demonstrated that pimavanserin (PVT) suppresses pancreatic tumor growth by inducing autophagy-mediated apoptosis. Our results indicated that PVT induced apoptosis and reduced the proliferation of pancreatic cancer cells with IC_{50} ranging between 3-9μM after 24, 48 and 72 hours of treatment. In addition, PVT inhibited the colony formation of pancreatic cancer cells. Treatment of pancreatic cancer cells with increasing concentrations of PVT resulted in a concentration-dependent increase in autophagy as evaluated by acridine orange assay by flow-cytometry. PVT induced the expression of autophagy markers ULK1, FIP200, Atg101, Beclin-1, LC3B and p62 in a concentration-dependent manner in several pancreatic cancer cells. Apoptotic effects of PVT in pancreatic cancer cells was validated by increase in cleavage of caspase3 and PARP. Oral administration of PVT suppressed BxPC3 tumor xenografts by 50% in athymic nude mice. In another *in vivo* experiment, PVT treatment inhibited the growth of orthotopically implanted PANC1 tumors by 75%. Autophagy was confirmed in the tumors of PVT treated mice by western blotting. Apoptosis was confirmed in the tumors of PVT treated mice by immunohistochemistry and western blotting. Chronic administration of PVT did not exhibit any general signs of toxicity or behavioral side effects in mice. Moreover, long-term administration of PVT did not alter the clinical chemistry parameters like ALT, AST, total serum protein, calcium and albumin. Collectively, our results indicate that PVT mediated pancreatic tumor growth suppression was associated with the induction of autophagy and apoptosis. Since PVT is already available in the clinic with an established safety profile, our results will accelerate its clinical development for pancreatic cancer therapy.

2723 Cytotoxicity of Peracetic Acid Vapor Exposures on Human Bronchial Epithelial Cells

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Peracetic acid (PAA) is a highly reactive peroxygen compound that is widely used as a disinfectant in healthcare settings and poultry processing plants. PAA is irritating and corrosive to the eyes, skin, and mucous membranes of the respiratory tract. Workers exposed to PAA vapors from the spraying and fogging of disinfectant solutions have reported symptoms such as headaches, lacrimation, coughing, wheezing, and blurred vision. Using a commercially available solution of PAA (32 weight % in dilute acetic acid), we aimed to identify the possible cytotoxicity associated with vapor exposures on normal human bronchial epithelial cells (NHBEs) *in vitro*, differentiated on porous, transwell membranes at the air-liquid interface. Using an in-house exposure chamber, NHBEs were exposed to filtered air (FA) for 1 hour (h), 2 h, and 4 h. This was to account for differences in temperature, relative humidity (RH) and carbon dioxide (CO_2) as compared to the cell culture incubator. Temperature in the chamber was 74.4 °F, RH was 90.4%, and CO_2 in ambient air was 0.04%. Using the water soluble tetrazolium assay, no significant differences in viability were detected between FA-exposed cells and incubator controls. Next, NHBEs were exposed to either FA (controls), 12 or 22 ppm of PAA for 4 h and then returned to incubators for an additional 4 or 24 h recovery period. Cellular viability, lactate dehydrogenase (LDH) production, cytokine production (IL-1β, IL-6, IL-8, and TNFα) and microscopic changes in cells were assessed at 4 and 24 h post exposures. Compared to FA controls, PAA (22 ppm) reduced cellular viability and increased LDH release, denoting acute cytotoxicity upon exposure at both time points. Cytokine production in FA-treated cells was not significantly different than PAA-treated cells, except for IL-6 at both time points. Microscopic changes were assessed using H&E staining of cells. Compared to controls at 24 h, PAA caused cell necrosis and loss of cilia. Our studies show that exposure of NHBE cells to PAA causes significant changes in viability, LDH production, IL-6 production and microscopic abnormalities when compared to FA. Further studies are needed to delineate the exact mechanism(s) by which this occurs.

2724 Development of a Highly Accurate Genome Sequencing Method and Its Application in the Genome-Wide Analysis of Chemical Mutation Signatures

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Direct detection of rare and mutagen-induce*d mutations* using a genome sequencer will facilitate a more accurate understanding of the mutational profile of mutagens and their relationships with cancer. However, this is difficult due to a higher frequency of errors associated with the existing genome sequencers. Thus, we developed a highly accurate genome-sequencing method utilizing double-strand DNA sequence and analyzed genome-wide mutations caused by exposure to chemicals. We optimized our sequencing method and achieved high accuracy with an error frequency of less than one per 10^7 -10^8 bp. We analyzed DNA samples of mutagen (i.e., *N*-methyl-*N*-nitrosourea (MNU), *N*-ethyl-*N*-nitrosourea (ENU), diethylnitrosamine (DEN), benzo[a] pyrene (B[a]P), and aristolochic acid (AA))-exposed *Salmonella* typhimurium TA100 cells and *gpt* delta mice, and obtained large-scale genome-wide somatic mutation data. In *gpt* delta mice, approximately 2500-63000 mutations per material were detected depending on the materials. Meanwhile, in *S. typhimurium*, approximately 1300-95000 mutations per material were detected. The six-type mutational spectra showed clear mutation patterns that represented the action mechanisms of each mutagen (e.g., G:C > A:T for MNU). In ENU or AA-exposed samples, significantly different mutational patterns were obtained from *S. typhimurium* and *gpt* delta mice, which suggested the differences in mutagenesis between these species. In MNU-exposed, but not ENU- or DEN-exposed *gpt* delta mice, the 96-trinucleotide mutation signature analysis revealed patterns similar to a known signature of an alkylating agent in human cancer (i.e. signature 11 in the COSMIC database). Furthermore, the signatures for ENU and DEN exhibited a high similarity with each other, suggesting that these mutagens have a common mutagenic mechanism. These results indicated that our sequencing method is useful for precisely characterizing chemical mutagenicity and establishing the link between mutagens and cancer.

2725 Development of a Novel Genome-Wide Mutagenicity Assay Based on Highly Accurate Genome Sequencing: A Case Study Using *Salmonella typhimurium* TA100

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We developed a highly accurate genome sequencing method that comprehensively detects rare mutagen-induce*d* mutations. To evaluate its applicability and utility, we analyzed DNA samples of *Salmonella* typhimurium TA100 exposed to mutagens. We evaluated 12 mutagens of various structural groups, including those requiring metabolic activation (*i.e.* alkylating agents, aldehydes, aromatic amines, and polycyclic aromatic hydrocarbons (PAHs)), and assessed the detection limit of mutation frequency and the ability to analyze various mutation patterns. Our method detecte*d* mutations caused by all 12 mutagens, including those with low mutation frequency such as aldehydes (c.a. 7-fold increase compared to the control), which induced nearly 2-fold increase in the number of His^+ revertants compared to control in Ames test. Although PAH-induce*d* mutations were detected, the sensitivity was not sufficient to assess mutagenic mechanisms. TA100 may not have been sufficiently exposed to these mutagens in the liquid culture due to their extremely low water solubility. Therefore, we modified the exposure conditions for these chemicals. For this, TA100 was allowed to form His^+ revertants on agar plates post mutagenic exposure. Then, genomic DNA was extracted from these cells for mutational analyses. Thereby, 7,12- dimethylbenz[a]anthracene- and 3-methylcholanthrene-induce*d* mutations on the major spectrum (G:C > T:A) increased by ~5.4 and 4.1 times relative to liquid culture samples. Therefore, our method can sensitively detect most TA100-positive mutagens. Furthermore, mutagens from the same structural group exhibited similar mutation spectra (*e.g.* G:C > A:T for alkylating agents), which reflected the mutagenic mechanism of each group. Next, we evaluated the 96-trinucleotide mutational signatures using the obtained mutational data. Signatures of the alkylating agents (*e.g. N*-methyl-*N*-nitrosourea) were similar. Similarly, those of aldehydes, aromatic amines, and PAHs were similar to each other (broad spectra of G:C > T:A). These signatures were also similar to known mutational signatures of alkylating agents and tobacco in human cancer, respectively (*i.e.*, signature 11 and 4 in COSMIC database). Our method provides detailed data on mutagen-induce*d* mutations and promotes understanding of their mechanism of action and relationship with human cancer.

2726 Development of an Intracellular Microtubule Stability Assay Using GFP-α-Tubulin

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Aneuploidy, the presence of an abnormal number of chromosomes, is often observed in cancer and several classes of chemicals are known to cause aneuploidy. To detect aneugenicity as result of chemical exposure, typically the micronucleus assay is used. However, both broken DNA fragments caused by clastogenic agents and mis-segregated chromosomes caused by aneugenic agents can lead to the formation of micronuclei. To confirm an aneugenic mode of action, a laborious centromere or FISH staining is needed to assess whether broken chromosomes or complete chromosomes are present in the micronucleus. Aneugenicity can be caused by any process that interferes with chromosome segregation during mitosis, including microtubule disruption or inhibition of cell cycle kinases like Aurora A/B. Here we established an assay to study the effect of substances on tubulin stability, to provide more insight into the cause of aneuploidy upon substance exposure. Several *in vitro* and cellular assays are available to measure tubulin stability. However, most of these assays are dependent on a tubulin labelling step on fixed cells. Here we developed an assay using GFP-tubulin, allowing the direct visualisation of microtubuli, using either live cell microscopy to follow the dynamics during the cell cycle or flow cytometry. First a GFP-Tubulin reporter was stably integrated into mouse embryonic stem cells. We confirmed that the GFP-tubulin was properly integrated in microtubuli. To measure the effect of microtubule disrupting substances, cells were treated with various tubulin poisons and the amount of GFP-tubulin signal in microtubules was quantified using flow cytometry. A DNA staining was included to assess the effect of the agent on the cell cycle distribution. In the assay, both tubulin stabilising and tubulin destabilising substances could be detected. Treatment with both types of substances resulted in an accumulation of cells in G2/M phase. Treatment with agents that affect cell cycle progression but not microtubule stability, such as DNA damaging agents or Aurora kinase inhibitors, did not affect tubulin stability. In conclusion, we developed a microtubule stability assay in mouse embryonic stem cells that can efficiently detect both stabilising and destabilising microtubule disruptors and can be used to detect an aneugenic mode-of-action of genotoxic compounds.

2727 The Impact of Structural Modification of Oxaliplatin on Cell Survival

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The platinum compounds cisplatin, carboplatin, and oxaliplatin are FDA approved cancer treatments in the US These platinum compounds are cell-damaging agents with comparable structures that are used for the treatment of different cancer types. They each have two ligands attached to the platinum atom: a leaving and a non-leaving ligand, both of which vary across compounds. Despite the diversity of platinum-based compounds, the mechanism of cytotoxicity is similar, triggering apoptosis by interrupting replication, the specific compound to be used in treatment is determined by the tissue of origin. We hypothesize that the structural differences in these novel compounds will alter cell-specific cytotoxicity and intracellular accumulation, as compared to oxaliplatin. In this study, two novel compounds, derivatives of oxaliplatin were tested for toxicity in several cell lines. Oxalato((1R,2R)-N,N-dimethyl-1,2-diaminocyclohexane)platinum(II) (Pt(me2dach)(ox)), differs from oxaliplatin as in that a hydrogen on each nitrogen of the non-leaving ligand has been replaced with a methyl group. Oxalato(S,S)-1,2-diaminocyclohexane (Pt(S,S-dach)(ox)), differs from oxaliplatin only in the stereochemistry of the leaving ligand. Both novel compounds have an oxalate leaving group identical to oxaliplatin. The toxicological profile of our novel compounds was determined in cell lines derived from embryonic kidney cells (HEK 293), melanoma cells (SK-Mel-5), and colon cancer cells (HT-29). The cell lines are individually plated at equal concentrations at time 0, then at 24 hours exposed to increasing concentrations of the platinum compound. After a total of 48 hours, an MTT assay is performed to determine cell survival. From the survival concentration-response curve, the IC50 value is determined. The difference in the cell survival between the cell types is established by comparison of the IC50. Pt(me2dach)(ox) has lower cell death than oxaliplatin in melanoma (SK-Mel-5) and preliminary data suggests the same trend in embryonic kidney cells (HEK293). Pt(me2dach)(ox) causes higher cell death in HEK 293 cells than in SK-Mel-5. Concerning the novel platinum Pt(S,S-dach)(ox), the comparison of the IC50 values between HEK293 and HT-29 show an increased cell survival at higher concentrations in the HT-29 cells. This compound also showed a lower IC50 than oxaliplatin in HEK293.

2728 Induction of Aromatase Expression in Breast Cancer Cells through a Non-Genomic Signaling of Estrogen Receptor Alpha 36

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Estrogens play an important role in the development and progression of breast cancer. In addition, estradiol has been show to modulate aromatase expression in breast cancer cells. The enzyme responsible for the production of estradiol is aromatase, a member of the cytochrome P450 family. The effects of estrogen are mainly mediated by their interaction with the estrogen receptor (ER). 17β-Estradiol (E2) has reported to induce the development and growth of various ER-negative breast cancers. The effects are rapid and non-genomic, suggesting that a membrane-associated ER (mER) is involved. ERα36 has been shown to mediate rapid, non-genomic, membrane-associated effects of E2 in several cancer cell lines, including triple negative MDA-MB-231 breast cancer cells. The aim of this study was to determine if ERα36 mediates aromatase expression, and to elucidate the mechanism involved. Treatment with BSA conjugated E2 (BSA-E2)-induced aromatase protein expression in human breast cancer cells; enhancing aromatase gene expression, and promoter activity. Treatment with G15, an estrogen receptor antagonist, did not affect the inductive effects of BSA-E2 on aromatase expression. In addition, BSA-E2 increased the phosphorylation of Akt, ERK and CREB in their signaling pathways in human breast cancer cells. ERα36-silencing was used prior to these treatments to determine the role of ERα36 in these effects and to determine which signaling molecules were involved. We found that ERα36 in fact mediates the increased aromatase expression by BSA-E2 in breast cancer cells. Therefore, these results suggest that estradiol promote to aromatase expression through ERα36-mediated phosphorylation of Akt, ERK and CREB. In addition, it is provide that ERα36 may be a potential target for drug design against breast cancer, particularly triple negative breast cancer.

2729 *In Vitro* Assessment of the Transactivation of Chicken Estrogen Receptor by Bisphenol Analogs

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Bisphenol A (BPA) has been used for the manufacture of polycarbonate plastics and epoxy resins. BPA and its analogs (bisphenols; BPs) are widely detected in the environment, and their potential estrogenic effects on wild animals are of great concern. It is known that some of BPs elicit estrogen-like responses. However, little information is available for the effect of BPs on the estrogen receptor (ER) signaling pathway in birds. There are also still unanswered questions about the structural preference of BPs by ER and the molecular mechanism of ER activation. To assess the transactivation potency of BPs *via* chicken ERα (ckERα), we measured the potencies of 26 bisphenol analogs (BPs) by using the *in vitro* reporter gene assay system that ckERα was transiently expressed in COS-1 cells. Results showed that 4,4′-methylenebisphenol (4,4′-BPF), 2-[(4-hydroxyphenyl)methyl]phenol (2,4′-BPF), bis(4-hydroxyphenyl)methanone (HBP), and 4,4′-thiobisphenol (TBP) had dose-dependent responses, whereas 4,4′-(1-methylethylidene)bisphenol (BPA), 4,4′-[2,2,2-trifluoro-1-(trifluoromethyl)ethylidene]bisphenol (BPAF), 4,4′-ethylidenebisphenol (BPE), 2,2′-methylenebisphenol (2,2′-BPF), 4,4′-sulfonylbisphenol (BPS), 4,4′-(2,2-dichloroethenylidene)bisphenol (BPC2), 4,4′-(1-phenylethylidene) bisphenol (BPAP), and 1,1′-(1-methylethylidene)bisbenzene (DPP) had no transactivation potency. Relative potencies (E_2-REP_{20}) estimated from relative effective concentrations (REC_{20}) of tested BPs that induce the response corresponding to 20% of the maximum 17β-estradiol (E_2) response indicated that HBP had the highest E_2-REP_{20} (5.0×10^{-4}), followed by 2,4′-BPF (1.0×10^{-4}), 4,4′-BPF (7.3×10^{-5}), and TBP (6.1×10^{-5}).

2730 Regulation of ARNT Alternative Splicing upon T Cell Activation

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The aryl hydrocarbon receptor (AHR) is a cytosolic receptor that mediates the effects of environmental contaminants on the body. When activated, AHR translocates to the nucleus and dimerizes with the aryl hydrocarbon receptor nuclear translocator (ARNT) and together they bind to xenobiotic response elements of AHR-ARNT related genes. Early studies have shown that AhR-deficient mice have altered lymphocyte numbers in the spleen, thus showing the importance of AHR for homeostasis of the immune system. In fact, AHR has been found to be functionally up-regulated during naïve T cell activation and promotes the expression of the pro-inflammatory cytokine IL-22. This up-regulation of AHR and IL-22 is essential to allow T cells to respond and promote an inflammatory response when exposed to environmental contaminants like tobacco smoke. The immune system requires a high degree of diversity to adapt and respond to these changes in the environment. Upon T cell activation, about 10-15% of alternative exons undergo a >10% change in inclusion which is critical for eliciting an immune response. The main binding partner of AHR, ARNT is alternatively spliced to produce two main isoforms, ARNT isoform 1 and 3, which differ in the inclusion of exon 5 found in ARNT isoform 1. Interestingly, naïve lymphocytes have equal amounts of ARNT isoform 1 and 3, whereas during T cell activation there is an increase in the amount of ARNT isoform 1. We have identified that RBFOX2, an RNA binding protein up-regulated during T cell activation, as a key regulator of ARNT alternative splicing. Targeted suppression of RBFOX2 in a T cell cancer line showed a decrease in the amount of ARNT isoform 1 with an increase in isoform 3. Site-directed mutagenesis was used to map RBFOX2 binding to specific sequences in the downstream intron which promotes the inclusion of ARNT exon 5 into the final mRNA transcript. Through targeted suppression of either ARNT isoforms it was found that the loss of ARNT isoform 3 promoted an increase in expression of the crucial T cell activation cytokine IL-2. This study has identified a novel role of how ARNT is alternatively spliced to produce an isoform that is up-regulated after naïve T cell activation and is critical in promoting a proper T cell response to adapt to environmental changes.

2731 Regulation of Co-regulator Recruitment by an AhR-Activation-Directed, CK2-Mediated Phosphorylation of ARNT Isoform 1

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The aryl hydrocarbon receptor (AhR) is a cytoplasmic bound receptor that is activated by interaction with various endogenous or exogenous ligands. Once activated, AhR translocates to the nucleus where it associates with the aryl hydrocarbon receptor nuclear translocator (ARNT) to form a functional DNA binding heterodimeric transcription factor, which is involved in numerous physiological processes including toxicant responses, immunological function, and development. Interestingly, ARNT is alternatively spliced into two distinct isoforms, ARNT isoform 1 and ARNT isoform 3. These two ARNT isoforms differ by 15 amino acids that make up exon 5 of ARNT isoform 1. This exon encodes a unique phosphorylation site at serine 77 (S77), which can be robustly induced upon exposure to both exogenous and endogenous AhR ligands. Recently, we have determined that ARNT rapidly undergoes phosphorylation in human T cell lines following AhR activation, however the function of this phosphorylation event remains unclear. We hypothesized that upon AhR ligand exposure, ARNT undergoes phosphorylation that is mediated by casein kinase 2 (CK2) to perform a crucial role in AhR target gene transcription and co-regulator recruitment, thus allowing for a specific toxicant response. We have determined that ARNT isoform 1 phosphorylation is dependent on AhR activation as the inhibition or knockdown of AhR results in a significant reduction in phosphorylation compared to samples with intact AhR function. We have also found that upon inhibition and knockdown of CK2, the phosphorylation event of ARNT isoform 1 is abolished and correlates with a significant decrease of AhR target gene transcription and a decrease in recruitment of the well-documented co-regulator, CREB binding protein (CBP), while not effecting the interaction of ARNT with AhR. These data suggest that ARNT isoform 1 phosphorylation serves to promote a more robust and, possibly, a specific AhR signaling response.

2732 Evaluating the Effects of PCB 126 on Hepatic Energy Metabolism in AhR Knockout Mice

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Epidemiologic studies have shown that exposures to polychlorinated biphenyls (PCBs) are associated with liver toxicity including nonalcoholic fatty liver disease (NAFLD). Based on their structure and ability to activate the aryl hydrocarbon receptor (AhR), PCBs are classified as dioxin-like (PCB126) or non-dioxin-like. Previously, our group demonstrated that acute exposures to low doses of PCB126 caused hepatic and pancreatic toxicity in male mice and upregulated AhR target genes. Because the AhR regulates a numerous physiological and pathological processes, the current study aims to examine if the observed PCB 126 hepatic/pancreatic effects are mediated primarily through AhR activation. Male C57BL/6J and $AhR^{-/-}$ mice were obtained from Taconic Biosciences and exposed to low-dose PCB 126 (20 µg/kg) by gavage. After 2 weeks, mice were euthanized, and blood and tissue samples were collected for histology and other downstream analyses. Compared to wildtype C57BL/6J mice, $AhR^{-/-}$ mice had lower liver weight/body weight ratio, higher white adipose tissue/body weight ratio and higher body fat composition. Importantly, $AhR^{-/-}$ mice demonstrated hepatic lipid accumulation (steatosis) as demonstrated by histological analysis (H&E, Oil Red O staining). $AhR^{-/-}$ mice also showed higher levels of hepatic free fatty acids and triglycerides, and PCB 126 exposure exacerbated this effect. In contrast, $AhR^{-/-}$ mice had lower plasma triglyceride and cholesterol levels, implicating systemic dyslipidemia. Additionally, AhR ablation altered hepatic gene expression resulting in upregulation of lipogenic genes (CD36, FASN) and downregulation of fatty acid oxidative genes including PPARα. Glucose tolerance test demonstrated that $AhR^{-/-}$ mice had lower fasting blood glucose and insulin levels, while PCB 126 exposure decreased area under the curve, irrespective of genotype. $AhR^{-/-}$ mice also exhibited increased pancreatic weight relative to body weight. Finally, AhR deficiency altered hepatic expression of other PCB receptors, including upregulating pregnane-xenobiotic receptor and downregulating constitutive androstane receptor, suggesting xenobiotic receptor cross talk. Taken together, the results implicated that AhR plays a pivotal role in maintenance of lipid metabolism and energy homeostasis, and suggest that PCB126 may act through non-AhR pathways to trigger hepatic toxicity.

2733 Isoflavones as Ah Receptor Agonists in Colon-Derived Cell Lines: Structure-Activity Relationships

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Many of the protective responses observed for flavonoids in the gastrointestinal track resemble aryl hydrocarbon receptor (AhR)-mediated effects. Therefore, we examined the structure-activity relationships of isoflavones and isomeric flavone and flavanones as AhR ligands based on their induction of CYP1A1, CYP1B1 and UGT1A1 gene expression in colon cancer Caco2 cells and young adult mouse colonocyte (YAMC) cells. Caco2 cells were significantly more Ah-responsive than YAMC cells and this was due, in part to flavonoid-induced cytotoxicity in the latter cell lines. The structure-activity relationships for the flavonoids were complex and both response and cell context-specific, however, there was significant variability in the AhR activities of the isomeric substituted isoflavones and flavones. For example, 4′,5,7-trihydroxy isoflavone (genistein) was AhR-inactive whereas 4′,5,7-trihydroxyflavone (apigenin) induced CYP1A1, CYP1B1 and UGT1A1 in Caco2 cells. In contrast, both 5,7-dihydroxy-4-methoxy substituted isoflavone (biochanin A) and flavone (acacetin) induced all three AhR-responsive genes; 4′,5,7-trimethoxyisoflavone was a potent AhR agonist and the isomeric flavone was AhR-inactive. These results coupled with simulation studies modeling flavonoid interaction within the AhR binding pocket demonstrate that the orientation of the substituted phenyl ring at C-2 (flavones) or C-3 (isoflavones) on the common 4-H-chromene-4-one ring strongly influences the activities of isoflavones and flavones as AhR agonists.

2734 Effects of *In Utero* and Lactational Exposure of Mice to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototypical environmental contaminant in the class of dioxin-like chemicals. Studies by others have reported that mice with keratinocyte-specific constitutively active aryl hydrocarbon receptor (AHR) develop atopic dermatitis (AD)-like symptoms during their lifetime. In this study, we investigated the effects of perinatal exposure to TCDD, a potent AHR ligand, on the development of epidermal barrier and susceptibility to AD-like skin condition in C57BL/6J mice. Mice were exposed *in utero* to TCDD at a dose of 5 µg/ kg bw on embryonic day 12 and the effects on barrier formation and function were studied from postnatal day (PND) 1 through adult life stages. Pups exposed to TCDD were born with diffuse epidermal hyperplasia; however, this effect did not persist in adult life stages. The animals grew with no visible signs of eczema and had normal levels of IgE between PND 35-135. Because TCDD is immunosuppressive, we used a vitamin D analogue (MC903) to induce AD-like inflammation in skin. No significant differences in response to MC903 were observed between TCDD-exposed and the control animals. Animals in both groups exhibited visible skin inflammation with epidermal hyperplasia and high levels of serum IgE, indicating a normal Th2 response. Compared to control animals, levels of CYP1A1 RNA and protein were increased in TCDD-exposed mice. Levels of CYP1A1, CYP1A2, and CYP1B1 were highest at PND 13-21, then decreased to control levels by PND 70. While CYP1A2 and CYP1B1 were expressed throughout the epidermis, CYP1A1 was localized at the infundibulum of hair follicles, junctional zone and sebaceous glands. Histological analysis (H&E and Oil Red O staining) showed signs of sebaceous hypoplasia. Overall, perinatal exposure of mice to TCDD results in a chloracne-like pathology of the epidermis, without evidence of atopy.

2735 AH Receptor Ligand-Specific Modulation of Prostaglandin D_2 Metabolism in Human Keratinocytes: Evidence for a Critical Involvement of the Epidermal Growth Factor Receptor

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Prostaglandin (PG) D_2 is released by mast cells and plays an important role in the development of cutaneous allergic reactions and atopic dermatitis (AD) by activating CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) and stimulating Th2 cell-driven immune responses. PGD_2 either spontaneously hydrolyzes to the anti-inflammatory PPARγ agonist 15Δ-PGJ_2 or is reduced by aldo-keto reductase (AKR) 1C3 to 9α,11β-PGF_2. The latter metabolite is less potent in activating CRTH2 than PGD_2 but metabolically stable, thus prolonging cutaneous allergic inflammation. By using LC-MS analysis, we here provide evidence that exposure of human HaCaT keratinocytes to the polycyclic aromatic hydrocarbon (PAH) benzo(a)pyrene (BaP), but not to the dioxin-like polychlorinated biphenyl (PCB) 126, fosters the 11-ketoreduction of exogenous PGD_2 to 9α,11β-PGF_2 in an aryl hydrocarbon receptor (AhR)- and AKR1C3-dependent manner. Further studies revealed that, in contrast to PCB126, BaP treatment induced AKR1C3 expression through an epidermal growth factor receptor (EGFR)-dependent non-canonical AhR signaling pathway. Both AhR agonists, however, activated the soluble tyrosine kinase c-Src, stimulated phosphorylation of the metalloproteinase ADAM17 and the associated ectodomain shedding of cell surface-bound EGFR ligands. Surprisingly, this release of EGFR ligands stimulated EGFR activity only in BaP- but not in PCB126-treated cells. By means of EGFR internalization assays using Alexa Fluor™ 555-conjugated EGF, we provide evidence that PCB126, but not BaP, is capable of displacing EGF from the extracellular domain of EGFR, thus acting as a potent inhibitor of EGFR activity. Taken together, this study identifies (1) an inhibition of EGFR by PCB126 as being causative for the observed AhR ligand-specific effects on AKR1C3 expression and 9α,11β-PGF_2 formation, and (2) a potentially clinically relevant mechanism by which PAHs, but probably not dioxin-like PCBs, may exacerbate the course of cutaneous allergic eruptions and chronic inflammatory skin diseases, such as AD.

2736 Understanding the Relationship between the Aryl Hydrocarbon Receptor (AhR) and the Translocator Protein (TSPO) in Regulating Mitochondrial Function in Mouse Lung Epithelial Cells

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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor and part of the Per-Arnt-Sim (PAS) superfamily of environmental sensors. The AhR mediates most, if not all, of the toxicity induced by certain environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In contrast to TCDD-induced toxicity, the AhR also plays a role in normal developmental and homeostatic processes, presumably upon binding to putative endogenous ligands, such as cholesterol, heme, and tryptophan derivatives. Interestingly, studies suggest that a portion of the cellular pool of AhR resides within the mitochondria, though its function in this organelle is not defined. Another important mitochondrial protein is the translocator protein (TSPO). TSPO was identified several decades ago, however, its cellular function is not understood. TSPO is also known to interact with cholesterol and heme derivatives, similar to the AhR. Given their shared location (i.e. in the mitochondria) and ability to bind similar metabolites, we hypothesized that crosstalk between the AhR and TSPO regulates mitochondrial metabolism and the efficiency of the electron transport chain (ETC). To test this hypothesis, we measured the oxygen consumption rate (OCR) and mRNA expression of critical genes via quantitative real time polymerase chain reaction in wild type mouse lung epithelial cells (i.e. MLE12s) and MLE12s that are null for the AhR and TSPO. Our mitochondrial stress test results suggest that crosstalk between AhR and TSPO modulates ETC efficiency as we observed changes in our WT cells we did not see in the $AhR^{-/-}$ and $TSPO^{-/-}$ cells. Also, upregulation of IDO1 and IDO2 in WT cells treated with AhR and TSPO ligands further supports our hypothesis.

2737 Free Fatty Acids Receptor 1/4 (FFAR1/4) Agonists, GW9508, and TAK875 Attenuate Agonist-Induced Shortening in Human Airway Smooth Muscle (HASM) Cells

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Asthma is an airway disease characterized by airway hyper-responsiveness, inflammation and remodeling. Enhanced contractile phenotype of airway smooth muscle (ASM) cells mediates bronchoconstriction in asthma. Obesity is associated with asthma development, severity and response to treatments. The mechanisms linking obesity and asthma are yet to be determined. Free fatty acids receptors (FFARs) are G-Protein Coupled Receptors (GPCRs) and are emerging as crucial signaling molecules with importance in metabolic and inflammatory diseases like asthma. Therefore, we hypothesized that the free fatty acid receptors FFAR1 and FFAR4 modulate cell shortening in HASM cells. In our experiments, HASM cells were treated with vehicle (DMSO) or synthetic FFAR1/4 agonists, GW9508 or TAK875 (0.1 - 10 μM), for a short duration (5, 10, 20, or 30 min), followed by stimulation with contractile agonists carbachol (CCh, 10 μM) or histamine (2.5 μM). Myosin light Chain (MLC) phosphorylation and agonist-induced cytosolic Ca^{2+} ($[Ca^{2+}]_i$) were determined as a surrogate measure of ASM cell shortening. In parallel experiments, histamine-induced cellular stiffness was measured in HASM cells by magnetic twisting cytometry (MTC). To determine whether ASM relaxation signaling is altered by FFAR1/4 agonists, isoprenaline-induced cyclic AMP (cAMP) levels were measured in HASM cells in the presence of vehicle, GW9508 or TAK875. Both GW9508 and TAK875 significantly attenuated agonist-induced MLC phosphorylation in HASM cells in a concentration and time-dependent manner. Histamine-induced HASM cell stiffening was similarly reduced by GW9508 and TAK875, although the decrease was not statistically significant. FFAR1/4 agonists had little effect on agonist-induced $[Ca^{2+}]_i$ or isoprenaline-induced cAMP levels in HASM cells. Our findings show that FFAR1/4 agonists, GW9508 and TAK875, attenuate agonist-induced HASM cell shortening by inhibiting MLC phosphorylation. FFAR1/4 may be novel therapeutic targets to broncho-protect human airways in airway diseases such as asthma and COPD.

2738 Ligand Activation of Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) Inhibits Growth of Human Prostate Cancer Cell Lines

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Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) mediates complex roles in many cellular processes, including lipid and glucose homeostasis, cell proliferation, differentiation, and inflammation. However, the role of PPARβ/δ in carcinogenesis remains controversial due to inconsistent results in the literature. To critically examine the role of PPARβ/δ in human prostate cancer tumorigenicity, two stable cell lines overexpressing PPARβ/δ were generated with a MigR1 retroviral vector system. Both an androgen receptor (AR) positive and AR-negative cell lines were created using LNCaP and PC3 cells, respectively because previous evidence by others suggested that AR-positive prostate cells increased proliferation following ligand activation of PPARβ/δ whereas this effect was not observed in AR-negative prostate cancer cells. Expression of PPARβ/δ was markedly higher in MigR1-hPPARβ/δ LNCaP and PC3 cells as compared to parent and vector controls. The expression of the PPARβ/δ target gene angiopoieten-like protein 4 (*ANGPTL4*) in MigR1-hPPARβ/δ LNCaP and PC3 cells was significantly higher in response to a specific PPARβ/δ ligand as compared to controls. Ongoing efforts are assessing the effects of ligand activation of PPARβ/δ in these cell lines on anchorage-dependent and anchorage-independent clonogenicity and tumorigenicity using both *in vitro* and *in vivo* approaches. Preliminary results from these studies indicate that ligand activation of PPARβ/δ inhibits growth and potentially tumorigenicity and that higher expression of PPARβ/δ is associated with inhibitory effects in a prostate cancer model, regardless of expression of AR. This result is similar to effects observed in estrogen receptor (ER)-positive and ER-negative breast cancer cell lines.

2739 The Effect of Selenium Isosteric Replacement on the Efficacy of a PPARβ/δ Ligand in a Human Melanoma Cell Line

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The persistent low survival rate of melanoma patients requires the search for new therapeutic approaches for this disease. Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) may be a suitable molecular target for this purpose due to its reported ability to inhibit melanoma carcinogenicity. Interestingly, selenium (Se) can inhibit tumorigenesis and its isosteric substitution for sulfur (S) in compounds can increase the potency/efficacy of cancer therapeutics. The present study examined the efficacy of the PPARβ/δ ligand GW501516 and its selenium analog Se-GW501516 in a human melanoma cell line (UACC903). Both PPARβ/δ ligands similarly inhibited proliferation of UACC903 cells. Over-expression of PPARβ/δ caused greater sensitivity to this effect, as Se-GW510516 led to reduced cell proliferation sooner than GW501516. Whereas neither ligand modulated clonogenicity in control cells, both ligands inhibited clonogenicity in UACC903 cells over-expressing PPARβ/δ. Both ligands activated PPARβ/δ as shown by an increase in the PPARβ/δ target gene angiopoietin-like 4 (*ANGPTL4*) in UACC903 control cells. These effects were greatly enhanced in UACC903 cells over-expressing PPARβ/δ. There was no change in the mRNA expression of the putative PPARβ/δ target gene 3-phosphoinositide-dependent protein kinase 1 (*PDPK1*), a known modulator of the protein kinase B (AKT) pathway. Finally, the ability of ligand activation of PPARβ/δ to modulate ultraviolet (UV) radiation-induced cysteine-aspartic proteases-3/7 (CASPASE-3/7) activity was examined. All cell lines increased CASPASE-3/7 activity in response to UV irradiation; UACC903 cells over-expressing PPARβ/δ exhibited a heightened response. Whereas neither PPARβ/δ ligand modulated protease activity after 4h administration, a 24h pre-treatment with either ligand increased CASPASE-3/7 activity compared to controls in UACC903 cells over-expressing PPARβ/δ. Collectively, these studies provide further evidence that PPARβ/δ expression and/or ligand activation inhibited proliferation and clonogenicity, and enhanced apoptosis in UACC903 melanoma cells. Isosteric substitution of S for Se only modestly influenced these endpoints, but further studies are needed to assess novel approaches to targeting PPARβ/δ as a cancer therapeutic.

2740 Effect of Activating Retinoic Acid Receptor (RAR) and Peroxisome Proliferator-Activated Receptor-β/δ (PPARβ/δ) in a Human Melanoma Cell Line

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New targets for melanoma are needed due to low survival of patients with this disease. High expression and/or ligand activation of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) can inhibit carcinogenicity of a human melanoma cell line (UACC903). All-trans retinoic acid (atRA), which activates retinoic acid receptors (RARs), is postulated to activate PPARβ/δ and increase expression of 3-phosphoinositide-dependent protein kinase 1 (PDPK1) and protein kinase B (AKT). This study examined whether atRA and a PPARβ/δ ligand (GW0742) could influence PPARβ/δ activity in UACC903 cells. UACC903 cells cultured with atRA exhibited reduced proliferation and over-expression of PPARβ/δ conferred greater sensitivity to this effect. Expression of the RAR target gene cytochrome P450 26A1 (*CYP26A1*) was increased by atRA in UACC903 cells but over-expression of PPARβ/δ did not influence this effect. By contrast, the mRNA expression of the PPARβ/δ target gene, angiopoietin-like 4 (*ANGPTL4*), or *PDPK1* were unchanged in response to atRA in both UACC903 control and PPARβ/δ over-expressing cells. Co-treatment of UACC903 cells with atRA or GW0742 inhibited proliferation as compared to controls, but no synergy or additivity in this effect was observed. Whereas ligand activation of PPARβ/δ inhibited anchorage-dependent clonogenicity in cells over-expressing PPARβ/δ, co-treatment with atRA did not impact this effect. Expression of *CYP26A1* mRNA was elevated in response to atRA in UACC903 control cells and those over-expressing PPARβ/δ compared to controls. Expression of *ANGPTL4* mRNA was increased by ligand activation of PPARβ/δ in control UACC903 cells and this effect was enhanced in UACC903 cells over-expressing PPARβ/δ; no synergy or additivity were observed. In contrast, the mRNA expression of *PDPK1* was unchanged by either ligand activation of RAR or PPARβ/δ, or both. Collectively, these studies show that UACC903 cells over-expressing PPARβ/δ are sensitive to the anti-proliferative effects of either an RAR or PPARβ/δ ligand. The latter could be due in part to enhanced activity of the PPARβ/δ heterodimerization partner RXR. Co-treatment with an RAR or PPARβ/δ ligand did not cause any additional PPARβ/δ activities. These studies provide further evidence that atRA does not activate PPARβ/δ or the PDPK1/AKT pathway in a human melanoma cell line.

2741 Ciglitazone, a Human PPARγ Agonist, Disrupts Dorsoventral Patterning in Zebrafish

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Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor that regulates lipid/glucose homeostasis and adipocyte differentiation. While the role of PPARγ in adipogenesis and diabetes has been extensively studied, little is known about PPARγ function during early embryonic development. Within zebrafish, maternally-loaded ppaγ transcripts are present within the first 6 h post-fertilization (hpf), and *de novo* transcription of zygotic ppaγ commences at ~48 hpf. Since maternal ppaγ transcripts are elevated during a critical window of cell fate specification, the objective of this study was to test the hypothesis that PPARγ regulates gastrulation and dorsoventral patterning during zebrafish embryogenesis. To accomplish this objective, we relied on 1) ciglitazone as a potent PPARγ agonist and 2) a splice-blocking, ppary-specific morpholino to knockdown ppaγ. We found that initiation of ciglitazone - a potent human PPARγ agonist - exposure by 4 hpf resulted in concentration-dependent effects on dorsoventral patterning in the absence of epiboly defects during gastrulation, leading to ventralized embryos by 24 hpf. Interestingly, ciglitazone-induced ventralization was reversed by co-exposure with dorsomorphin (DMP), a bone morphogenetic protein (BMP) signaling inhibitor that induces strong dorsalization within zebrafish embryos. Moreover, mRNA-sequencing revealed that lipid- and cholesterol-related processes were affected by exposure to ciglitazone. However, ppaγ knockdown did not block ciglitazone-induced ventralization, suggesting that PPARγ is not required for dorsoventral patterning nor involved in ciglitazone-induced toxicity within zebrafish embryos. Our findings point to a novel, PPARγ-independent mechanism of action and phenotype following ciglitazone exposure during early embryonic development.

2742 Unexpected Induction of *cyp3a65* mRNA in Zebrafish Harboring a CRISPR-Mutated Pregnane x Receptor

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More than half of all pharmaceuticals used by humans are metabolized by the CYP3A4 enzyme and most vertebrates express a CYP3A ortholog. Pregnane x receptor (PXR; NR1I2) is a nuclear receptor that regulates transcriptional responses to drug or xenobiotic exposure, including activation of *CYP3A* transcription. PXR is a promiscuous receptor, responding to a wide range of ligands that differ across species and make functional studies on its role in the chemical defensome most relevant when approached in a species-specific manner. In zebrafish embryos, the ligand pregnenolone has been shown to activate Pxr leading to induction of *cyp3a65* and *pxr* transcription that can be abrogated through microinjection of translation-blocking morpholinos that inhibit Pxr production. Genetic knockout studies in rodents targeting *Pxr* support a critical role for PXR activation of *CYP3A* expression, however genetic knockouts in zebrafish have not previously been explored. Here, we report on two different zebrafish lines created by CRISPR-mediated gene editing. One line harbors a 107 bp deletion in exon 2 that includes coding sequence for Pxr's DNA binding domain and results in a frameshift and subsequent early termination codon that are detectable in the expressed transcripts. The second line is characterized by a 37 bp deletion in exon 7 and total deletion of intron 7 and exon 8, with the expressed transcript showing a direct splicing of exon 6 to exon 9. Notably, exons 7 and 8 contribute coding sequence for translation of the ligand binding domain. The obvious prediction for these mutations is a loss of functional gene products. To our surprise, larvae homozygous for either of the *pxr* mutant alleles retain their ability to induce *cyp3a65* mRNA expression averaging 1.9-fold enrichment. Additionally, auto-induction of *pxr* expression follows the same pattern observed in wildtype fish, with *pxr* mRNA expression averaging a 2.6-fold enrichment in response to pregnenolone exposure. These data suggest a compensatory mechanism may be responsible for *cyp3a65* and *pxr* induction. Two alternative possibilities are that Pxr is not required for the pregnenolone effect or that the mutated alleles produce truncated yet functional protein. To further investigate the above possibilities, we are currently testing two newly created CRISPR mutants with targeted deletions in exons 3 or 6. It is crucial that we develop a better understanding for the role of Pxr in this important biomedical test species. *Support for these experiments comes from NIH P42 ES007381 and R21HD073805 (JVG), Swiss National Science Foundation P2EZP2_165200 (NRB).*

2743 Assessment of Receptor Occupancy via Flow Cytometry: Benefits and Pitfalls of Two Common Approaches

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Receptor occupancy assays can be a powerful tool to determine drug dosage. Recently, flow cytometry has been the method of choice to assess receptor occupancy due to its ability to measure drug-target binding interactions on multiple cell populations simultaneously. Receptor occupancy assays using flow cytometry involve measurement of target receptors bound by the drug, number of receptors not bound to the drug, and the total number of receptors present. The methods for measuring these parameters, particularly for antibody-based therapeutics, can be categorized into two approaches based on the detection antibodies used. Here, we discuss the benefits and pitfalls to these methods. In one approach, fluorescently labelled competing and non-competing antibodies to the drug were used to detect free and total receptors respectively. In this approach, the competing antibody is often the labelled drug itself. One of the main obstacles in method development is to identify a non-competing antibody with comparable binding affinity to the target as the drug tested. We observed that when competing and non-competing antibodies display different affinities to the target, the results can be difficult to interpret. In addition, it is imperative that in a multiplex assay, competing and non-competing antibodies do not interfere with each other's binding to the target. The other approach utilized a single, fluorescently labelled secondary antibody to detect both bound and total receptors. Samples from animals treated *in vivo* were first incubated with excess drug *ex vivo* to fully saturate all targets, then stained with secondary antibody to detect total receptor. Staining with a single-detection antibody ensured that a direct comparison could be made between bound and total receptors, and removed challenges related to different binding affinities between reagents. Identifying a suitable secondary antibody was crucial, especially when the targeted populations consist of sticky cells such as granulocytes and monocytes. In addition, we found that incubation of samples *ex vivo* with excess drug can lead to downregulation of the target, leading to underestimation of the total receptors and overestimation of receptor occupancy of the drug. The technical considerations presented will aid to determine the most suitable approach and avoid common pitfalls in assay designs when incorporated early in the RO design phase.

2744 The Aryl Hydrocarbon Receptor Undergoes Chaperone-Mediated Autophagy in Triple Negative Breast Cancer Cells

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The aryl hydrocarbon receptor (AhR) is a well-known transcription factor which regulates many essential biological functions. Human breast cancers express high levels of AhR, suppression of this receptor function alters breast cancer cell growth, suggesting a plausible approach of targeting AhR for treatment of breast cancers. We discovered that a compound called Q18 - (4- (5-ethylpyrimidin-2-yl)piperazin-1-yl)(4-((quinazolin-4-ylamino)methyl)phenyl)methanone - acted as an AhR antagonist and promoted AhR protein degradation via the chaperone mediated autophagy (CMA) pathway in triple-negative, but not in non-triple-negative, breast cancer cells. In triple-negative MDA-MB-468 cells, Q18 induced complete and irreversible lysosomal degradation of AhR in 24 hours. Notably, blockage of CMA by epigenetic silencing of lysosomal-associated membrane protein 2A (LAMP2A), a key CMA effector that translocate cytosolic client proteins across the lysosomal membrane, protected AhR from the Q18-mediated protein degradation. Co-immunoprecipitation and proximity ligation assay demonstrated the physical interaction between AhR and LAMP2A. In addition, activation of the CMA pathway by known methods, such as treatment of 6-amino-nicotinamide and long-term nutritional deprivation, decreased the AhR protein levels in MDA-MB-468 cells. Collectively we have provided evidence supporting that the AhR protein can be degraded via CMA in triple-negative breast cancer cells.

2745 Knockout of the Aryl Hydrocarbon Receptor Increases Pancreas Cancer Lethality

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Only 10% of those diagnosed with pancreatic ductal adenocarcinoma (PDAC) live beyond 5 years. These dismal outcomes reflect a poor understanding of PDAC pathogenesis hindering efforts to develop more effective PDAC therapies. Recent discoveries suggest the Aryl Hydrocarbon Receptor (AhR) plays a crucial role in the pathogenesis of several cancers through mediation of effector immune cell infiltration. The AhR is a ligand activated transcription factor involved in many cellular processes, including regulation of immune function in the GI tract, liver and skin. Although there is some data suggesting a putative tumor suppressor function in PDAC, the extent AhR suppresses pancreas cancer development has not been well studied. For this reason, we sought to evaluate the effect of AhR loss in the development of PDAC *in vivo* using a novel mouse model. A widely accepted PDAC model, where the primary PDAC *Kras* mutation is present in pancreas lineage cells (KC mice) was crossed with AhR null ($A^{-/-}$) mice to explore the effects of AhR heterozygosity ($A^{+/-}$KC) and loss of AhR ($A^{-/-}$KC) on PDAC formation. At 9 months, rate of survival, grade and incidence of pancreatic intraepithelial neoplasia (PanIN) and PDAC are evaluated in KC, $A^{+/-}$KC, $A^{-/-}$KC and the control groups. To date, 30 KC and 17 $A^{+/-}$KC mice have been evaluated. So far, these mice develop a range of pathology (PanINs and PDAC) at about the same rates. In each group, 100% of mice had chronic pancreatitis and PanIN 1 lesions, the earliest form of neoplasia. Both KC and $A^{+/-}$KC mice have shown a 7% incidence of PanIN 2 lesions, while PanIN 3, the highest grade lesions, have only been seen in KC mice (7%). Additionally, PDAC was seen in 16% and 18% of KC and $A^{+/-}$KC respectively. This data suggests no histopathologic difference exists in pancreas between KC and $A^{+/-}$KC mice. Interestingly, a dramatic decrease in survival of the $A^{-/-}$KC mice compared to the KC and $A^{+/-}$KC groups has been found. The $A^{-/-}$KC mice (N=26) have a 35% survival rate compared to the 70%, and 66% survival rate of KC (N=44) and $A^{+/-}$KC (N=86) respectively. Due to this high mortality of the $A^{-/-}$KC mice, of the 26 that have been produced, only 2 have made it to 9 months for pathologic evaluation. Of these 2 mice, both had chronic pancreatitis and PanIN 1, 1 had PanIN 2 while neither had a PanIN 3 or PDAC. Although early, it appears loss of AhR may exacerbate the progression of PDAC given the higher mortality in the $A^{-/-}$KC. This suggests AhR may play a protective role in PDAC development and warrants further investigation.

2746 Aryl Hydrocarbon Receptor (AhR) Ligands Are Estrogen Receptor Alpha Degraders in Breast Cancer Cells

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Estrogen receptor alpha (ERα, ESR1) expressing breast cancers initially respond to endocrine therapy, however later stage ER-positive tumors develop drug resistance and second-line therapies including selective ER degraders (SERDs) are being developed. These drugs directly bind ERα and induce their degradation. It has previously been shown that ligand dependent inhibitory aryl hydrocarbon receptor (AhR)-ER crosstalk is due, in part, to activation of proteasome-dependent degradation of ERα. Initial studies showed that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) decreased ERα expression in MCF-7 breast cancer cell lines, and we also observed that some AhR ligands including 1,1-bis (3'-indolyl) methane (DIM) and the microbiome metabolite 1,4-dihydroxy-2-napthoic acid (DHNA) also decrease expression of ERα protein in MCF-7 cells. In contrast other microbial-derived AhR ligands including tryptamine, indole, indole-3-acetate and indole-3-pyruvate did not affect ERα expression. Moreover, cotreatment of MCF-7 cells with TCDD, DIM and DHNA with the proteasome inhibitor MG132 resulted in partial inhibition of AhR ligand-dependent ERα degradation demonstrating that ERα degradation by selected AhR ligands was proteasome-dependent. Approximately 40% of late stage metastatic ER-positive breast tumors express ER mutations Y537S or D538G in the ligand binding domain of ERα and MCF-7 cells expressing these mutants have been generated by gene editing technologies. Treatment of mutant MCF-7 (Y537S) or MCF-7 (D538G) cells with AhR ligands showed that both DIM and DHNA also induced proteasome-dependent degradation of ERα in MCF-7 cells expressing the mutant receptor. Thus, selective AhR ligands represent a new class of ERα degraders and current studies on the mechanism of AhR/ligand-mediated ERα degradation are ongoing.

2747 Role of the Aryl Hydrocarbon Receptor (AhR) in Mediatiing the Effects of Coffee in the Colon

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Coffee is the most widely consumed beverage worldwide and epidemiology studies show that there is an association between coffee consumption and health benefits including protection from Parkinson's disease, metabolic disease, several cancers and decreased rates of mortality. In addition, coffee drinkers are protected against inflammatory bowel disease and colon cancer and since these responses are similar to effects observed for the aryl hydrocarbon receptor (AhR) and its ligands we hypothesized that coffee extracts are AhR-active. Commercially available ground roasted coffee was extracted with hot water and the water extracts were also extracted with chloroform and both the water and chloroform extracts induced 3 AhR-responsive genes, namely CYP1A1, CYP1B1 and glucuronosyltransferase 1A1 (UGT1A1) in Caco2

cells. In contrast coffee extracts did not induce gene expression in Caco2 AhR knockout cells (Caco2-AhRKO) and extracts of ground unroasted coffee beans were AhR-inactive. Subsequent fractionation studies showed that caffeine was AhR-inactive and norharman and related β-carboline were identified as one of the AhR-active components in the coffee extract. Coffee extracts also inhibited growth of intestinal stem cell enriched organoids *in vitro* and this response was not observed in organoids derived from mice in which intestinal AhR is silenced (Villin^{Cre}AhR$^{fl/fl}$). We also observed that coffee extracts inhibited dextran sodium sulfate (DSS) induced barrier function damage *in vivo* in mice and coffee extract also inhibited DSS-induced expression colon mucosal interleukin-6 (IL-6), IL-10, IL-10 receptor1, interferon-γ and transforming growth factor β. Coffee extracts also modified gut microbial metabolites. These data suggest that the health benefits of coffee extracts in the intestine are associated with the AhR activity of the extract.

PS 2748 Modulation of Ligand-Mediated Aryl Hydrocarbon Receptor Activity Influences HNSCC Resistance to Nutritional and Chemotherapeutic Stress

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Head & Neck Squamous Cell Carcinoma (HNSCC) recurrence following clinical intervention represents a major obstacle to effective treatment and survival rates. A key feature of tumor recurrence is the capacity of tumor cells to adapt to stresses associated with the nutrient-limited tumor microenvironment and toxic chemotherapeutic agents. Aryl Hydrocarbon Receptor (AHR) expression and transcriptional activity has been previously shown to influence cell cycle progression and pro/anti-apoptotic signaling, among other aspects of HNSCC aggressiveness, implicating the AHR in mediation of tumor cell survival. However, the mechanisms by which AHR promotes HNSCC cell resilience or whether AHR antagonism can restrict HNSCC survival have not been fully explored. Both 2D monolayer and 3D spheroid culture approaches were utilized to examine stress responses and survival in HNSCC exposed to AHR agonists and antagonists. Clonogenicity-survival assays and MTT viability assays revealed a significant increase in HNSCC resilience to either nutritional (restricted nutrient) or chemotherapeutic-mediated (5-fluorouracil) stress in a series of HNSCC lines (HN30, HN2095, OSC19) upon exposure to AHR agonists. In contrast, AHR antagonists prompted a significant decrease in cell survival under stress conditions. Additionally, AHR-mediated changes in resilience was observed during clonogenicity-survival and viability assays conducted in 3D HNSCC spheroid cultures, more accurately representing the conditions of the tumor microenvironment. Exposure to AHR agonists prior to experimental stressors preconditioned HNSCC cells with enhanced expression of stress markers of the unfolded stress response (ATF4, XBP1, CHOP), nutrient transporters (SLC7A5/3A2), nutrient sensing (WARS), drug transporters (ABCC5), and modulators of cell cycle progression (CDKN1A), while AHR antagonists reversed such preconditioning. Activation of the AHR contribute to HNSCC adaptation and survival in the context of nutritional or 5-fluorouracil-mediated stress. Conversely, antagonism of AHR activity severely restricts HNSCC resilience *in vitro*. Such data reveal AHR expression and transcriptional activity as an important adaptive component resulting in enhanced HNSCC resilience, thus implicating AHR antagonism as a potential therapeutic approach to limit HNSCC recurrence.

PS 2749 Effects of Monocyte Chemoattractant Protein-1, Macrophage Inflammatory Protein-1α, and Interferon-α2a on P450 Enzymes in Human Hepatocytes *In Vitro*

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Cytokines response to oligonucleotide drug tilsotolimod can reveal the potential for interactions between the immunomodulator and co-administered small molecules. Tilsotolimod, an agonist of Toll-like receptor 9, stimulated the release of macrophage chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α) interferon gamma-induced protein 10 (IP-10), tumor necrosis factor alpha (TNF-α) and interferon-α2a (INF-α2a) in blood obtained from healthy donors. Although tilsotolimod did not directly affect CYP1A2, 2B6 or 3A4 m RNA expression or activity, the cytokines stimulated by the drug reduced CYP2B6 and CYP1A2 enzyme activities in cultured human hepatocytes (1). Number of the cytokines stimulated by tilsotolimod remain uncharacterized for their potential to modify expression of drug metabolizing enzymes. This study sought to identify which cytokines were responsible for tilsotolimod effects on P450 enzymes *in vitro*. Primary human hepatocytes from three donors were cultured in sandwich configuration in the modified Chee's medium supplemented with 10% normal human plasma. Cells were treated with MCP-1 (2, 10 or 50 ng/mL), MIP-1α (0.4, 2 or 10 ng/mL) or INF-α2a (0.1, 0.5 or 2.5 ng/mL) with dosing solutions prepared fresh daily for three days of treatments. These concentrations were 0.2-, 1- or 5-fold the concentration found in plasma stimulated with tilsotolimod (1). Measurements of the levels of selected mRNAs and enzyme activities were described elsewhere (2). Recombinant chemokines MCP-1 and MIP-1α did not alter CYP1A2, 2B6, 2C8, 2C9, 3A4 or signal transducer and activator of transcription 1 (STAT1) mRNA or CYP1A2, 2B6 or 3A4/5 enzyme activity in co-cultures of human hepatocytes and Kupffer cells. INF-α2a, at 2.5 ng/mL, reduced CYP2B6 enzyme activity to 46% of control and increased CYP1A2 and STAT1 mRNA by 2.4- and 5.2-fold, respectively. This study established that INF-α2a, but not MCP-1 or MIP-1α, mediated tilsotolimod effects on CYP2B6 expression in human hepatocytes. 1. Tarantino P, et al, (2018) Cytokine-Mediated Suppression of CYP Enzymes By the Toll-like Receptor 9 Agonist, IMO-2125, in Cultured Human Hepatocytes. Hepatology 68:1444A 2. Czerwinski M, et al, (2015) Anti-CD28 monoclonal antibody-stimulated cytokines released from blood suppress CYP1A2, CYP2B6, and CYP3A4 in human hepatocytes *in vitro*. Drug Metab Dispos 43:42-52.

PS 2750 Bempegaldesleukin (NKTR-214), a Novel IL-2 Based Immunotherapy, Demonstrates Superior Nonclinical Safety Compared to That Reported for Recombinant Human IL-2 (rhIL-2)

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Bempegaldesleukin (BEMPEG), a novel immunotherapeutic prodrug comprising IL-2 conjugated with polyethylene glycol (PEG), is currently in clinical trials with combination with check point inhibitors for the treatment of advanced cancers. Once administered, the prodrug releases active IL-2-PEG-conjugates that preferentially activate CD122 (the IL-2 receptor beta subunit), resulting in preferential expansion of CD8+ T cells over regulatory T cells in the tumor microenvironment. BEMPEG produces superior antitumor activity compared to recombinant human (rh)IL-2 in several mouse syngeneic tumor models when combined with check point inhibitors[1]. Safety of BEMPEG was evaluated in repeat intravenous (IV) dose toxicity studies in rats and cynomolgus monkeys for up to 15 weeks with an 8-week recovery, at doses up to 0.3 mg/kg in rats and 0.1 mg/kg in monkeys based on IL-2 content once every 2 weeks for 8 doses. Elevated white blood cells, predominately absolute lymphocyte counts, observed in both species are consistent with the expected pharmacology of IL-2. Primary toxicities associated with BEMPEG were inflammatory cell infiltrates, related to expected pharmacology, in multiple organs in both species with partial or complete recovery. Compared to the published nonclinical toxicity profile of rhIL-2[2], BEMPEG resulted in no new toxicities and fewer toxicities overall. Significantly, in contrast to previous reports for rhIL-2, there was no indication of vascular leak syndrome after administration of BEMPEG at the highest non-severely toxic dose in the nonclinical studies. Consistent with nonclinical studies, IV administration of BEMPEG in cancer patients, who previously received multiple anti-cancer treatment, was well tolerated and these patients demonstrated significant elevated absolute CD4+ T cell, CD8+ T cell, and NK cell counts[3]. Out of 26 evaluable patients, 9 patients (35%) had tumor shrinkage and 14 patients (54%) had stable disease. Such desirable outcome triggered the combination therapy development of BEMPEG with check point inhibitors in cancer patients and the clinical trials are still ongoing[4]. [1] Charych DH et al. Clin Cancer Res. 2016, 22(3):680-690. [2] Anderson TD et al. Int Rev Exp Pathol. 1993, 34 Pt A: 57-77. [3] Bentebibel SE et al. Cancer Discov. 2019, 9(6): 711-721. [4] Cancer Discov. 2019, 9(6): OF1.

PS 2751 Nonclinical Species Sensitivity to Convulsions: Survey Outcome of an IQ DruSafe Consortium Working Group Initiative

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Convulsions are considered severe adverse events, and compounds that carry a convulsion liability either require a large safety margin based on the most sensitive nonclinical species to advance to human clinical trials or will be limited by a strict clinical exposure cap. Within the pharmaceutical industry, dogs are perceived to be particularly sensitive to drug-induced convulsions.

To verify this perception, a survey was constructed within the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ, www.iqconsortium.org) and distributed amongst IQ participating pharmaceutical companies to compare nonclinical species for sensitivity to convulsions. The survey response contained blinded data on 80 compounds from 11 companies. Species sensitivity was assessed by comparing the lowest free drug plasma concentration at which convulsions were observed and the no observed effect level for convulsions between species. Data were also collected on other endpoints including use of the electroencephalogram, premonitory signs, convulsion type, the highest development phase reached and the reason why development was stopped. Key findings from the survey are: 1) the dog was most often determined to be the most sensitive species to convulsion, 2) there was no clear sensitivity ranking of other species, 3) no single reliable premonitory indicator of convulsion was identified, and 4) the lack of convulsions in human in this dataset suggests that convulsion liability is well mitigated with current drug development strategies.

PS 2752 Development of an *In Vitro* System Based on PXB-Cells for Evaluation of Mitochondrial Dysfunction-Mediated Hepatotoxicity

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Fresh human (h)-hepatocytes are considered the best *in vitro* model system for studying xenobiotic metabolism and hepatotoxicity. However, hepatocytes grown in two-dimensional cultures cannot be maintained for more than a week. We previously showed that h-hepatocytes (PXB-cells®) freshly isolated from chimeric mice with humanized livers (PXB-mice®) could be maintained as high-density culture (2.1×10^5 cells/cm^2) expressing major hepatic genes up to 3 weeks. In a conventional cell culture setup, hepatocytes are known to generate adenosine triphosphate (ATP), mainly via cytosolic glycolysis. Such anaerobically poised cells that impair mitochondrial functions are resistant to xenobiotics and are not suitable for the evaluation of mitochondrial toxicity. The susceptibility to mitochondrial impairment is increased by culturing hepatocytes in a galactose (Gal)-based medium, instead of a conventional glucose (Glu)-based medium, under hyperoxia conditions. In this study, we evaluated whether these improvements in culture conditions helped in assessing mitochondrial toxicity in PXB-cells. PXB-cells were cultured in the Gal-based medium under hyperoxic conditions in a multi-gas incubator. Results obtained were compared with those from PXB-cells cultured normally. PXB-cells in hyperoxic conditions (40% O_2) showed high sensitivity to rotenone compared to normal condition (20% O_2), as evaluated by LDH and intracellular ATP assays. PXB-cells are usually cultured in a low Glu-based medium, which is not remarkable different from the Gal-based medium. The oxygen consumption of cells, monitored by an oxygen concentration meter, was higher in the low Glu-based medium than in the Gal-based medium. PXB-cells were expected to exhibit high mitochondrial activity in the low Glu-based medium. Since the low Glu-based medium and the hyperoxic condition maintained the expression of several *CYP* mRNAs and CYP3A activity in PXB-cells equivalent to the normal condition, mitochondrial dysfunction induced by metabolites of a compound would also be checked in this condition. In conclusion, we established a mitochondrial hepatotoxicity assay using PXB-cells as an *in vitro* system.

PS 2753 Biocelerate Nonclinical Protocol Harmonization Initiative

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BioCelerate, a subsidiary of TransCelerate BioPharma, Inc., is a nonclinical industry consortium driving initiatives to increase efficiency and productivity in early-stage R&D. Our objective in developing common templates for nonclinical studies is to drive efficiencies in toxicology-study management across the spectrum of Biopharma and Contract Research Organizations (CROs). Our initial project resulted in a protocol template for FIH-enabling repeat-dose toxicology studies. The process to develop the initial protocol template utilized the OECD/FDA GLPs as a starting point and excluded process instructions where large variations in preferences existed. Advantages to using a common template include (1) decreasing the time it takes to develop a protocol and thus start a study, (2) improvement of overall study quality by decreasing errors due to unfamiliarity with protocol formatting, (3) optimization of time in managing multiple studies, and (4) optimizing time spent on subsequent report/SEND preparation and review. Two 2019 webinars introduced the initiative and were used to collect stakeholder feedback. Responses to poll questions indicated that a majority of stakeholders used multiple repeat-dose toxicology protocol templates, and experienced problems associated with protocol inconsistency including process/time inefficiencies and reduced quality of study execution. There was a mixed response regarding the inclusion of SEND information in the protocol. Building on these and earlier discussions, BioCelerate collaborated with CROs, health authorities, and BioPharma to develop a common protocol template for FIH-enabling repeat-dose toxicology studies. Version 1.0 of the protocol was released publicly in 4Q19 for voluntary adoption. The template and supporting implementation materials can be downloaded from the BioCelerate website. BioCelerate provides reports on the uptake/use of the common protocol template and shares critical feedback from early adopters on the gaps as well as the usefulness of the template. BioCelerate will continue to work with collaborators and stakeholders in evaluating options and recommendations for content and structural improvements. Input from all stakeholders will help us develop the next-generation protocol template as well as shaping our next-steps for subsequent common templates (e.g. Common Report Template). These activities will further support BioCelerate's goal of improving toxicology study operational efficiency and quality.

PS 2754 Induction of Malignant Lymphoma in Rats Given a Novel EZH2 Inhibitor for up to 13 Weeks

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Novel potent small-molecule epigenetic inhibitors of Enhancer of zeste homolog 2 (EZH2), a histone methyltransferase, are under consideration for development to treat solid tumors and lymphoma. Genetic- and pre-clinical data suggest potential pro-oncogenic effects of EZH2 inhibition based on the presence of EZH2 loss-of-function mutations in mouse models of pre-malignant myeloid conditions and development of hematopoietic malignancies in mice bearing a hematopoietic-specific deletion of EZH2. These findings have raised safety concerns of the carcinogenic potential early in the development of EZH2 inhibitors (EZH2i's). The FDA has recommended conducting a 13-week rodent toxicity study with EZH2i's earlier in development than required in ICH S9. To address this concern, in lieu of a 4-week toxicity study to enable a Phase 1 study in cancer patients, a novel potent orally bioavailable small molecule EZH2i was given once-daily for up to 13 weeks by oral gavage to Wistar Hanover rats (10/sex/group) at doses of 0 (vehicle), 5, 15, 30, and 45 mg/kg/day. At necropsy beginning as early as Day 58, thymic masses were observed along with increased thymus weights, and spleen enlargement at doses ≥15 mg/kg/day. These findings correlated with malignant lymphoma (12% incidence vs. 0.01% historical control rats of similar age) composed of neoplastic cells consistent with lymphocyte morphology, in thymus along with numerous hemolymphoreticular tissues (e.g. spleen, lymph nodes, etc.). Further evaluation of thymus and mesenteric lymph node for CD3 and CD79a expression (T-cell and B-cell markers, respectively) by immunochemistry (IHC), indicated that the lymphoma observed was predominantly composed of neoplastic lymphocytes positive for CD3 staining and negative for CD79a (i.e. T-cells derived). The data suggest that the lymphomas noted in this study are consistent with observations in mice with EZH2 loss-of-function mutations and/or EZH2 hematopoietic-specific knock-out. Overall, although further investigations are needed to confirm the carcinogenic mechanism, the data provide evidence confirming a risk for carcinogenicity following pharmacologic inhibition of EZH2.

PS 2755 Regulatory and Practical Perspectives on the Nonclinical Safety Testing in Support of Pediatric Medicine Development

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The Best Pharmaceuticals for Children Act became law in 2002 which has led to testing of drugs in pediatric population. In support of this, testing in juvenile animals has become a practice and an expectation. There has been significant discordance on the modalities of expectations of such studies from both regulatory and drug developers' perspectives. This has been captured in the latest 2018 ICH S11 Step 2 draft guidance. The purpose of this presentation is to review the evolution of such research, to share lessons learned (educational) and discuss authors' experience on regulatory interactions. To this end, pertinent literature was reviewed, expert opinions were sought, and authors' personal experiences are discussed considering the principles

of 3Rs (Replacement, Reduction, and Refinement). Results showed: in about 40% of cases, companies felt they had received discordant advice on such studies from the health authorities. Most juvenile toxicity studies have been performed due to lack of scientific knowledge or regulatory experience, or because there was a "perceived" uncertainty or risk, rather than a "truly" identified concern that needed to be addressed. ICH S11 guidance takes a more holistic view of the topic by ensuring that its focus is not limited to toxicological concerns alone, rather address pharmacology considerations, ensure such studies cover the relevant developmental stages of animals in relation to intended pediatrics, offer advice on the selection of appropriate endpoints, and such studies are required if they can add value. While designing such studies considerations on: selection of the best species (80% used rat and the rest used other rodents and nonrodents, based on scientific rationale), sponsors shall make a case for right animal age, dosing regimen and appropriate endpoints with a focus on not to "just add what you can do" rather "to do what you need to do". Authors' up to 10 interactions with regulators concurred this practice. Other considerations include: pediatric-first and pediatric-only indication where 2 species like the adult-setting, but in young animals with additional measures of developmental endpoints. It is concluded that new ICH S11 draft guidance captures the lessons learned, gives the option for sponsors to make a case. Such experiences were shared by the authors' regulatory interactions. These findings are of value to all stakeholders conducting such research to make a difference in those "waiting" pediatric patients.

PS 2756 AAV Vectors on the Move: Safety Studies in Nonhuman Primates for Second-Generation Gene Therapy Products

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The field of gene therapy has matured in recent years to bring forward potential treatment or cure for life-threatening monogenetic diseases. For *in vivo* gene delivery, a viral vector containing the gene of interest is administered and DNA/RNA is either directly inserted into the host genome (e.g. retroviral vectors) or is delivered via episomes in the nucleus of transduced cells (e.g. adeno-associated virus (AAV) vectors). AAV is by far the most commonly employed technology for gene delivery into cells. The first AAV-based gene therapy (GT) products are already available to patients in various indications and even more are undergoing preclinical and clinical development. The conduct of a clinical trial for an investigational GT product is guided by the Code of Federal Regulations (CFR) Title 21, Part 312 in the USA and by a variety of guidelines published by the European Medicines Agency in Europe. As with other medicinal products, preclinical pharmacology and toxicology studies need to support the decision that a clinical trial in human subjects is reasonably safe and scientifically feasible to conduct. Those studies are typically conducted in nonhuman primates (particularly the cynomolgus monkey), given that it shares > 98% homology to the human genome. This publication summarizes experience with a broad range of GT products that have been investigated in cynomolgus monkeys. It covers in total 125 animals, that were placed on 4 different studies, investigating 4 different GT products. One product was based on AAV5, two were based on AAV8, and one was based on AAV2/AAV8/AAV9. One product was administered into the *striatum* via convection-enhanced delivery, one was injected into the *cisterna cerebromedullaris*, one was administered below the *retina*, and one was injected intravenously. The publication describes selection of animals based on the presence of endogenous antibodies against AAV serotypes, outlines the prototypical design of NHP safety studies for such products, and summarizes the approach to determine distribution and transduction efficiency of these products.

PS 2757 Is Acidification of Urine a Preanalytical Requirement for the Measurement of Urine Calcium, Phosphorus, and Magnesium in Nonclinical Studies?

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Introduction: Measurement of urinary calcium (Ca), inorganic phosphorus (iPHOS) and magnesium (Mg) is often performed in nonclinical studies to monitor renal function and bone turnover in different laboratory species. To limit the potential underestimation of minerals present in urine, acidification is recommended to dissolve complexes formed by Ca and Mg cations with phosphate anions. Calcium oxalate crystals are commonly observed in rat and non-human primate (NHP) urine, and triple phosphate crystals are frequent in dog, hence sequestering a certain quantity of minerals. Although reducing the urinary pH is necessary to limit the formation of these crystals, calcium carbonate crystals may form in acidic urine in these species. We investigated the necessity of pre-analytical acidification in nonclinical urine samples in rats, dogs and NHPs. Methods: Urine samples were obtained from Cynomolgus monkeys, Beagle dogs and Sprague-Dawley rats during an overnight or morning collection. One aliquot of each sample was acidified by addition of HCl (1:10) to reach a pH ≤5.0, incubated for 30 minutes, centrifuged and analyzed. Urine Ca, iPHOS and Mg were measured on a Cobas® 6000 analyzer (Roche) on both, acidified and non-acidified samples. Results: The mean differences in urine concentrations between nonacidified and acidified samples in rats, dogs and NHP were 4.4%, 10%, 25% for Ca, 6.6%, 27%, 24% for Mg, and 3.4%, 11.2% and 58% for iPHOS, respectively. Large percentage differences were due the low mineral concentrations observed in NHP, and absolute concentration ranges remained similar between both methods. Ca and Mg concentrations were higher in acidified samples compared to non-acidified, while iPHOS concentrations were generally lower in monkey acidified samples compared to non-acidified. These differences were not statistically different and were not judged to exceed the biological variability. Conclusion: Acidification of urine did not result in noteworthy differences in the measurement of Ca, iPHOS and Mg, and was considered an unnecessary procedure in the context of nonclinical studies.

PS 2758 Toxicology Species Selection for Preclinical Safety Assessment of TLR7/8 Prodrug Agonist

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NKTR-262, a prodrug of TLR7/8 agonist (TLRX) conjugated to PEG via a releasable linker designed for slow release of TLRX to reduce toxicity, is in clinical development as an immuno-oncology drug. *In vitro* hepatocyte metabolism, cellular, and molecular studies, and *in vivo* measurements of target engagement demonstrated that rat and dog are relevant toxicology species. In human and dog peripheral blood mononuclear cells (PBMCs), TLRX induced comparable pERK1/2 phosphorylation levels with mean EC50s of 942 and 778 ng/mL, respectively. In contrast NKTR-262 failed to induce pERK1/2 in human cells; in dog cells induction occurred only at very high concentration. After 24 h stimulation of human, rat and dog PBMCs, TLRX induced dose-dependent IL-6 and TNFα secretion with similar potencies of 1.1 - 2.2 μM in dog and human cells and roughly 25-fold higher potency in rat cells at 0.05 μM. NKTR-262 elicited vastly less potent cytokine response in all 3 species consistent with a prodrug design. *In vivo* pharmacological responses to TLRX after single s.c. administration of NKTR-262 to rat (0.3mg/kg) and dog (up to 1 mg/kg) were evaluated by qRT-PCR measurement in blood cells for type I interferon and NFκB related gene expression (*MX1*, *Cxcl10*, and *Tnfa*) and induction of proinflammatory serum cytokines (IL-5, IL-6, IL-10, INFγ, TNFα and CXCL10 (IP-10)). In rat, expression of all 3 target genes was robustly induced. Amplitude of induction varied between 5- to 10-fold for *Tnfa* to ~2000-fold for *Cxcl10*. Expression of all 3 genes returned to near baseline levels by 48 h post dose. Rapid increase in all measured proinflammatory cytokines was observed in rat with serum levels returning to baseline by 48 h post dose. However, IP-10 showed substantially prolonged elevated levels at all dose levels which may be due to the high *Cxcl10* gene expression. In dog, NKTR-262 did not affect levels of IL-5, IL-6, IL-10, INFγ, and TNFα. IP-10 levels increased in most time points compared to baseline, however, due to variability within replicates and fluctuations within time points, no significant correlation could be attributed to NKTR-262 administration. TLR7/8 engagement by NKTR-262, and more potently by TLRX, was confirmed in rat and dog by *in vitro* and *in vivo* assessments. The type and extent of metabolites were consistent across human, rat and dog in *in vitro* hepatocyte assays. These results verify rat and dog as relevant toxicology species for the safety assessment of NKTR-262.

PS 2759 Evaluation of Rat Hepatoxicity of a GalNAc-Conjugated siRNA After Repeated Bolus Administration versus Continuous Infusion

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Subcutaneous injection (SC) of small interfering RNAs (siRNAs) conjugated to a trivalent *N*-acetylgalactosamine (GalNAc) ligand are an established tool for targeted hepatic delivery and subsequent degradation of target mRNAs. Screening of GalNAc-siRNA candidates has identified compounds that elicit hepatotoxicity at suprapharmacological doses in rats. Hepatotoxicity in rats has been hypothesized to result from RNAi mediated off-target effects, disruption of endogenous RNAi pathways, and/ or accumulation of siRNA and metabolites in target tissues. In this study, the test article, siRNA1, is a tool-kit siRNA-GalNAc conjugate directed against a rodent-specific liver-expressed mRNA target. siRNA1 was previously shown to produce hepatotoxicity in both the mouse and rat by a mechanism that is not related to pharmacodynamic

reduction of the target mRNA. The objective of this study was to determine if the hepatotoxicity observed with siRNA1 after three once weekly SC bolus doses over a two-week period could be mitigated by instead administering the equivalent cumulative dose via a continuous SC infusion. Male Sprague Dawley rats (n=4/group) were administered vehicle (0.9% NaCl), 40 mg of siRNA1, or 100 mg of siRNA1 via an implanted osmotic pump over a two-week period. The animals were sacrificed on Day 15. The analysis included clinical observations, assessment of body weight, organ weight, histopathology, and clinical pathology. One male at 40 mg siRNA1 and one male at 100 mg siRNA1 were found moribund on Day 14 and exhibited a > 15% loss of body weight compared to previous weekly weigh-in. Animals in the 40 and 100 mg dose groups displayed rough haircoat, thin appearance, hunched posture, or pilo-erection beginning on Day 13 and lasting until Day 15. Animals in the 40 mg and 100 mg siRNA1 dose groups had lower mean body weight gain compared to controls on Day 14. The histopathology and clinical pathology results following SC infusion were similar to those observed following repeated bolus administration of similar cumulative doses of siRNA1 suggesting that there was little to no mitigation of toxicity with continuous infusion.

PS 2760 Evaluation of the Toxicity Study by Using Cynomolgus Monkeys in *PiggyBac* Transposon-Mediated Chimeric Antigen Receptor T Cells

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Recently, the first CAR-T cell medicines were approved, and development of new CAR-T cell medicines targeting other blood and solid tumors is expected. For any new drug, it is necessary to conduct various nonclinical safety studies during development. However, CAR-T cell medicines are quite different from conventional pharmaceuticals. In nonclinical safety evaluation of CAR-T cell medicines, cytokine-release syndromes, on-target/off-tumor reactions, elimination of effects of heterologous GVHD, and dose setting of imported CAR-T cells require consideration. Therefore, it is necessary to establish 1) an autologous model in which donor and recipient are the same species, 2) a primate model of high antigen homology with humans, and 3) a large animal model allowing dose setting for nonclinical safety evaluation. To solve these problems, we engineered several CAR T-cell therapies using a non-viral piggyBac transposon system by electroporation and performed toxicity studies in cynomolgus monkeys with the support of the AMED (Japan Agency for Medical Research and Development) "Basic Technology Development Project for Industrialization of Regenerative Medicine and Gene Therapy". GMR CAR-T cells were produced from peripheral blood of cynomolgus monkeys using a GM-CSF receptor (GMR) CAR transposon vector and administered intravenously to each donor animal. Control animals received non-genetically modified T cells. Parameters included general condition, body weight, food consumption, hematology, blood chemistry, coagulation tests, TK analysis and cytokine profiles. Fourteen days after administration, all monkeys were euthanized for histopathology examinations. The obtained simian GMR CAR-T cells expressed anti-human GMR CAR and almost completely killed a human acute-myeloid leukemic cell line. GMR CAR-T cells were detected in all treated animals, but no abnormalities or postmortem findings were noted. These results show we successfully generated non-virally modified CAR T cells from cynomolgus monkeys, and more long-term studies using these CAR-T cells are planned.

PS 2761 Is It Hypertension or Are You Just Excited to See Me?

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In alignment with the 3Rs, and in an effort to maximize study endpoints, safety pharmacology (SP) parameters are being collected within the scope of toxicology (Tox) assessments with greater regularity. These additions serve to assess both acute and cumulative pharmacodynamic effects on the vital organ systems (CV, CNS, and respiratory) following administration of an investigational entity. While both disciplines (SP and Tox) share commonality to identify liabilities preceding clinical evaluation, these disciplines differ in the sensitivity with respect to acute changes in evaluated endpoints. As it is regulatory expectation that studies evaluating SP endpoints on Tox must maintain the same rigor to that of a stand-alone SP study, careful consideration should be given to study design and logistics. Considerations range from schedule of the evaluation intervals, to control of the data collection environment. To illustrate the sensitivity of SP endpoints, heart rate and blood pressure were evaluated in male beagle dogs and non-human primates (NHPs) following animal room entry to determine the potential impact on data interpretation. Data were evaluated to assess average impact (increase of SP parameters) following room entry (minute 1 following entry) and animal manipulation (clinical observation and/or blood draw). Control data were evaluated from 27 subjects over 7 studies (3 dog/4 NHP studies) to calculate maximal increases in each parameter and duration for the subjects to return to pre-entry. On average, heart rate (HR)) increased 18% (32 bpm) and 113% (96 BPM), and systolic blood pressure (SBP) increased 17% (21 mmHg) and 23% (39 mmHg), in NHPs and dogs, respectively. Additionally, the data supported these non-manipulative (exclusive of handling or sample collections) interruptions required an average of 30 and 35 min, for animals to return to pre-entry values, for NHPs and dogs, respectively. For procedural events requiring phlebotomy, this duration increased, on average, up to 73% (52 min interruption). The data support careful consideration for control of the collection environment to minimize alterations in cardiovascular responses to external stimuli. Additionally, procedural activities should be limited; specifically surrounding T_{max} in an effort to avoid confounding interpretation.

PS 2762 Evaluation of the Safety and Skin Irritancy of a Novel Anhydrous System: PermE8 Anhydrous Gel, W06 Anhydrous Topical Gel, and VersaBase Anhydrous HRT in Human Epidermis Model

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A novel anhydrous system that includes three proprietary bases: PermE8 Anhydrous Gel (PermE8), W06 Anhydrous Topical Gel (W06) and VersaBase Anhydrous HRT, has been developed for a broad range of dermatological and cosmetic application. PermE8 and VersaBase Anhydrous HRT are both for transdermal drug delivery, while VersaBase Anhydrous HRT is more specific for female hormone replacement therapy (HRT). Different from the other two bases, W06 is designed to use topically. With water activity below 0.6 (Aw<0.6), the anhydrous bases provide an unfavorable environment for microbial growth, are therefore allowed extended default beyond-use dates (BUDs) without compromising their drug delivery capabilities. The aim of this study was to evaluate the safety and skin irritancy potential of the anhydrous system. An *in vitro* reconstructed human epidermis model (Epiderm) that contains normal human-derived epidermal keratinocytes was used. PermE8, W06, and VersaBase Anhydrous HRT were applied and spread evenly on top of EpiDerm. Cell viability was determined by MTT assay after 1, 4, 17 and 24 hours of application. After 24 hours, viable cells were 94.78±1.28%, 89.34±2.11% and 51.66±2.73% in PermE8, W06 and VersaBase Anhydrous HRT treated Epiderms, respectively. Viabilities were all above the irritation classification threshold of 50%, suggesting these tested bases are non-irritants. The exposure time required to reduce cell viability by 50% (ET-50) was more than 24 hours for PermE8 and W06, 24 hours for VersaBase Anhydrous HRT, and 9 hours for 1% Triton X-100, which was a positive control and moderate-to-mild irritant. Based on the correlation between *in vivo* and *in vitro* irritancy response, the irritancy of a product with ET-50 of 24 hours is as mild as baby shampoo. In conclusion, none of the three bases in this novel anhydrous system showed signs of cell toxicity after 24-hour treatment. All bases are non-irritants, and the irritancy is milder than baby shampoo. This study provides safe options to physicians and compounding pharmacists when choosing bases for topical or transdermal drug delivery.

PS 2763 Neratinib Causes Persistent Damage and Decreases Drug-Metabolizing Enzyme in Gut

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Neratinib (NERLYNX®) is a pan-HER tyrosine kinase inhibitor newly approved by FDA in 2017 and its European counterpart in 2018 to treat adult patients with HER2-positive breast cancer, but the approval of neratinib has been controversial. Recent retrospective analysis showed that neratinib did not impact patients' quality of life based on questionnaire, while another cost-benefit analysis indicated that neratinib was not cost-effective, due to severe side effect and high economic cost. ExteNET trial, the phase III trial of neratinib showed that 96% of the patients taking neratinib experienced diarrhea. However, very few mechanism studies have been reported about neratinib-induced gastrointestinal (GI) toxicity. Hereby, we performed toxicity studies in mice to characterize the potential mechanism underlying such adverse effect. C57BL/6 J (female, 5-weeks old) mice were randomly separated into three groups A, B, C. Group A received vehicle while group B was orally dosed with 100mg/kg neratinib once daily for 18 days. Group C was dosed

with 100mg/kg neratinib for 12 days and switched to vehicle afterwards for 6 days. Intestine and liver were collected for further analysis. Our results showed that 12 days treatment of neratinib caused persistent histological damage in mouse GI tract, including intestinal ulcer, blunted villi in jejunum and ileum, and remarkable inflammatory infiltrate in colon. In addition, we found that the gene expression of Cyp3a11 was significantly reduced (over 90% decrease, $p<0.005$) in small intestine but not changed in liver, which is the major enzyme metabolizing neratinib. The gene expression of proinflammatory cytokines, for example TNF-α and IL-6, increased remarkably (over 5 folds, $p<0.05$) throughout the GI tract. Such persistent damages were not recovered even after 6 days without neratinib treatment. In summary, neratinib caused the release of proinflammatory cytokines and downregulated the expression of Cyp3a11. With lower level of Cyp3a11, there is likely neratinib accumulation in gut. It eventually leads to high risk of diarrhea. Our findings give new insight into the mechanism of neratinib-induced GI toxicity. It also questions the current perspective that neratinib does not affect the physical well-being of patients.

PS 2764 Chronic Subcutaneous Administration of 30% (w/v) Aqueous Sulfobutyl Ether 7-β-cyclodextrin (Captisol™) in Wistar Han Rats Causes Malignant Sarcomas at the Injection Site

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Cyclodextrins solubilize and stabilize drugs and improve drug delivery. A polyanionic variably substituted sulfobutyl ether of β-cyclodextrin [sulfobutyl ether 7-β-cyclodextrin (SBE-β-CD; Captisol™)] interacts well with neutral drugs to facilitate solubility and chemical stability, and is used in products approved by the Food and Drug Administration. Five groups of 60 male and 60 female Wistar Han rats were administered a dose volume of 3 mL/kg saline control (Group 1), 30% (w/v) SBE-β-CD in sterile water for injection, final pH 7.2±0.5 (vehicle control/Group 2), or SBE-β-CD combined with low, mid, and high doses of a test article (Groups 3-5) by once daily subcutaneous administration in a 2-year carcinogenicity study. Due to rapidly expanding subcutaneous tumors in all groups except the saline control, males were terminated early during Week 60 and females were terminated early during Week 83. The subcutaneous tumors were initially observed after approximately 9 months of dosing and required humane euthanasia after approximately 4-6 weeks of growth. The tumors consisted primarily of pleomorphic fibrosarcomas and fibrosarcomas and were attributed to chronic irritation in the subcutaneous tissue related to SBE-β-CD administration. There was no exacerbation of tumor development by the test article. SBE-β-CD-related non-neoplastic findings included cytoplasmic vacuolation of epithelial cells in the kidney and prostate, parathyroid chief cells, and macrophages in the subcutaneous injection site. Chronic subcutaneous administration of 30% (w/v) SBE-β-CD causes malignant sarcomas at the administration site that result in high mortality and preclude its use as a vehicle for subcutaneous administration in 2-year carcinogenicity studies in rats.

PS 2765 The Utility of hERG Inhibition Data in the Derivation of Pharmaceutical Occupational Exposure Limits

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The human ether-a-go-go (hERG) channel is a highly-expressed voltage-gated potassium (K^+) channel in the human heart. Specifically, the hERG channel conducts the delayed rectifier K^+ current (IKR) and reestablishes resting potential, a crucial component of cardiac action potentials. Inhibition of the hERG channel leads to a decrease in the outward flow of K+, delayed repolarization, and consequent QT prolongation. Because QT prolongation may lead to a fatal arrhythmia known as Torsades de pointes (TdP), pharmaceutical drug candidates that exhibit potent hERG channel inhibition often fail early in clinical trials. However, many drugs with both cardiac and non-cardiac indications proceed to market despite having hERG channel inhibitory activity through various mechanisms. We identified classes of drugs with hERG inhibitory activity and evaluated the relationship between their cardiac toxicity and published occupational exposure limit (OEL). A total of 58 cardiac drugs across four classes (class I, class II, class III, and class IV) and 104 drugs with non-cardiac indications (e.g., psychiatrics, antibiotics) with published hERG channel IC_{50} values were identified. Published OELs or ranges were identified for 23 cardiac drugs and 46 non-cardiac drugs. A clear correlation between hERG IC_{50} potency and OEL values was observed in cardiac drugs; specifically, drugs with less potent hERG IC_{50} values had correspondingly higher OEL values. However, there was no apparent relationship between the hERG IC_{50} and OEL for non-cardiac drugs. Interestingly, 19 drugs had both a potent hERG IC_{50} (<25 μM) and a contrastingly large OEL value (>50 μg/m^3). Together, these data indicate that cardiac effects are likely the most sensitive endpoint (i.e., point of departure) driving the OEL value for cardiac drugs. On the other hand, the therapeutic effect used as the most sensitive endpoint driving OEL value for non-cardiac drugs may not have fully considered potential cardiac effects. Thus, we present a framework for consideration of cardiac effects when deriving OEL values.

PS 2766 Survey of Spontaneous Clinical Observations in the Cynomolgus Monkey (*Macaca fascicularis*)

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The Cynomolgus Monkey (*Macaca fascicularis*) is routinely used in preclinical toxicity testing and clinical observations are one of the many parameters used to assess the potential toxicity of a xenobiotic during the conduct of *in vivo* toxicology research. During the course of the study the frequency of clinical sign assessment varies depending on the type of study but will usually be conducted at least once daily with control animals being evaluated at the same time. Clinical observations from control animals were compiled and evaluated from 66 studies to assess the occurrence of common observations. The animals were characterized by their source/vendor, study duration, and genders. All the animals were social housed in stainless steel cages equipped with a bar type floor and an automatic watering valve. Typical observations noted included assessments of animal activity, appearance, presence of masses, discharge, normality and consistency of the stool, and any other unusual observations. Data were presented in a concise database allows for easy access to this historical background data. Clinical signs with higher incidences (approximately or higher than 1%) were alopecia, scab, bruise, anogenital regions swelling, vaginal discharge, toenail missing, skin discolored, tail deviation, abrasion, swelling, digit complete/portion missing, tooth missing, and soft stool. Husbandry related clinical signs were considered as alopecia and scab. The comparison duration of dose administration, the incidences of alopecia increased and scab were reduced for both sexes. The incidence of alopecia in females were higher than that in males. Technical related (dosing or blood collection) clinical signs were considered as bruising, vomitus, regurgitation, and toe nail missing. Sporadic and transient abnormalities included tremors, inappetence, slight salivation, distended abdomen, dry skin, skin scaling, erythema (thorax/dorsal), increased respiratory rate, and rash (abdomen) were noted in one or two studies. Access to such data bases will enable and facilitate the toxicologist to put the observed clinical signs in perspective during the course of a toxicological study and will help identify those signs that are truly related to the administration of the test material.

PS 2767 Drug-Induced Seizures: Considerations for the Underlying Molecular Mechanisms

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Drug-induced seizure (DIS) liabilities pose a life-threatening risk both in drug development and to the general population, significantly contributing to compound attrition and new-onset non-epileptic seizures in patients, respectively. Broad spectrums of known chemicals have been previously associated with DIS risk, emphasizing the importance of central nervous system drug safety assessments across various drug classes (e.g., antidepressants, antipsychotics, antibiotics, and analgesics amongst others). However, despite its importance within drug safety testing, a full understanding of the molecular mechanisms underpinning DIS is often lacking. The etiology of DIS is understood to be a result of either a deficit in inhibitory (e.g., gamma aminobutyric acid) or elevation in excitatory (e.g. glutamate) signaling, although other neurotransmitter systems are also known to play a role. However, how this altered neuronal signaling occurs and how these changes interact with other non-brain receptor driven DIS-associated changes such as metabolic disturbances, electrolyte imbalances, altered drug metabolism and withdrawal effects are poorly understood. Here, we aim to summarize and discuss important molecular mechanisms which were identified in the initiation of DIS for several known drugs and/or drug classes associated with DIS. With a better understanding of the molecular mechanisms underpinning DIS, mechanism targeted *in vivo* or *in vitro* strategies may be applied both pre- and post-marketing to characterize and mitigate DIS-risk during drug development. Our data shows that susceptibility stratification based on GABA-related drug in-

duced seizure presents species differences in the following order Beagle dogs > Sprague-Dawley rats > Cynomolgus monkeys > Göttingen minipigs with a more than 2-fold difference between canines and minipigs.

2768 Interim Safety of Intrathecal AAV9/GAT1 Gene Therapy

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SLC6A1 gene encodes the highly conserved gamma-aminobutyric acid (GABA) transporter GAT-1, a protein crucial for GABAergic signaling in the central nervous system (CNS). A subset of patients presenting with rare forms of infantile encephalopathy with intellectual disability (ID) have been shown to be heterozygous for loss-of-function mutations in SLC6A1. Broad delivery the SLC6A1 gene across the CNS using adeno-associated virus type 9 (AAV9) to restore normal GABAergic signaling may prove to be an effective strategy to treat patients with SLC6A1 genetic disorder. While the safety profile of AAV9 has been well established in both preclinical and clinical models, potential toxicity of overexpression and/or non-neuronal expression of the GAT-1 transgene is of concern. Two vector designs with different promoters were used; one is a weak universal promoter (P1), while the other is a stronger, neuronal-specific promoter (P2). Postnatal day 28-35 wild type C57BL/6 mice received the maximum feasible dose (7.5 and $7.0x10^{11}$ vector genomes (vg) for P1 and P2 constructs, respectively), four-fold lower dose, or vehicle control (PBS + 5% sorbitol) via a 5 µL intrathecal (IT) lumbar injection. Six males and six females were included in each dose group. Animals were weighed and observed weekly for body condition for four weeks, and monthly thereafter. Submandibular blood draw was taken at three weeks post-injection for serum chemistry analysis. No adverse reactions were observed post-injection in any animals, and all treated animals appear healthy up to 12 weeks post-injection and have normal bodyweight. Serum chemistry results show that aspartate aminotransferase (AST), blood urea nitrogen (BUN), albumin (ALB), and creatine kinase (CK) levels are not statistically different from vehicle control animals. Total bilirubin (TBIL) was significantly lower in both high-dose groups, although there is no known pathology associated low TBIL. These results help support the safety of IT delivery of AAV9/hGAT1opt transgene with either promoter. These animals will be followed to a year post-injection, at which point tissue will be harvested and assessed by a trained pathologist. With additional safety and efficacy testing, these preclinical data could be used to support the application this gene therapy approach to patients suffering from SLC6A1 haploinsufficiency disorder.

2769 Toxicological Safety Assessment of Extractable and Leachable from Printing Inks for Post-marketing Change of Parenteral Drug Products

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Regulatory authorities in US and EU have increased scrutiny and focus on extractable and leachable compounds over last several years. The safety assessment should be specifically discussed in Modules for Toxicology Written Summary/Other Toxicity of the ANDA/NDA/PAS/CBE submission. Nonclinical review of extractable/leachable studies is FDA hot topic, but unsure where reviewers will focus. To address these regulatory concerns, companies are spending significant time assessing toxicity of extractable\leachable (E/L) compounds. The Label Materials (such as adhesive, paper, ink, varnish, etc) are defined as "no direct contact" materials Per USP <1661>, which do not come into direct physical contact with drug products. However, depending on the container permeability and the label application process on the containers, the label materials can be considered as "potentially interacting" components. The presentation will discuss E/L studies design for post-marketing change in printing inks, E/L study results by using different analytical techniques (LC/PDA/MS, GC/MS etc.). Several extractables were detected from the ink extractions by High Performance Liquid Chromatography with a Diode Array Detector and Mass Spectrometry Detector (HPLC/PDA/MS) and Gas Chromatography with flame ionization detector and Mass Spectrometry Detector (GC/FID/MS). The presentation will also discuss how to determine the Safety Concern Threshold (SCT), calculate the Analytical Evaluation Threshold (AET) for drug products, and conduct toxicity/safety assessment for extractables and leachables per PQRI, ICH M7 as well as USP <1664> for drug products.

2770 Development of Claudin-5 Modulators and Evaluation of Their *In Vitro* Permeation-Activity of Solutes across the Blood-Brain Barrier in a Mammalian Model

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Introduction: A current bottleneck in the development of central nervous system (CNS) drugs is the lack of drug delivery systems targeting the CNS. The intercellular space between endothelial cells of the blood-brain barrier (BBB) is sealed by tight junctions (TJs). The TJ seal prevents the paracellular diffusion of drugs into the CNS. Claudins (CLDNs), a 27-member protein family of tetra-transmembrane proteins, are the major structural and functional components of the TJ seal. Although the BBB is more permeable to small molecules in CLDN-5-deficient mice than in wild-type mice, CLDN-5 deficient mice died within 10 h after birth. It is still unclear whether CLDN-5 is a safety and efficient target for drug delivery to the brain. In the present study, we generated anti-CLDN-5 monoclonal antibodies (mAbs) and examined the effects of the mAbs on the BBB. Methods: We generated anti-CLDN-5 mAbs by immunizing mice and rats with a plasmid encoding human CLDN-5. The effects of the mAbs on the integrity of the TJ seal were investigated by using MDCKII transfectants expressing CLDN-5 and a commercially available BBB model composed of cynomolgus monkey brain microvascular endothelial cells. Results: Four mAbs targeting the extracellular loop domains (ECLs) of human CLDN-5 were isolated. All four mAbs specifically bound to CLDN-5 but not to the other CLDNs. All of the anti-CLDN-5 mAbs dose- and time-dependently decreased TEER in an *in vitro* BBB model. The anti-CLDN-5 mAbs dose-dependently increased the permeability of the model BBB to the fluorescein dye and fluorescein-conjugated dextran (Mw 4 kDa) without cytotoxicity. Injection of anti-CLDN-5 mAbs also enhanced the permeability to the fluorescein dye without apparent adverse effects. Conclusion: The anti-CLDN-5 mAbs are the first CLDN-5-specific binders. The present results suggest that CLDN-5 is a potential target for drug delivery to the brain and these binders may be a potential lead for the development of novel drug delivery systems targeting the CNS. *Acknowledgements: This work was supported partly by a grant of Research Support from Astellas (RS2018A000674); Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19H04468, 18K19400, 18H03190, 16K13044, and 24390042); a grant for Research on Development of New Drugs from the Japan Agency for Medical Research and Development (AMED); and grants from the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research [BINDS]) of AMED (JP19am0101077, JP19am0101084, JP19am0101090).*

2771 A Comparison of Environmental Assessment Requirements of New Human Drugs in the US and the EU

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Market authorization of new human drugs in both the US and the EU requires an assessment of potential environmental impacts. In the US, drug approval is overseen by the Food and Drug Administration (FDA), and an environmental assessment (EA) is required pursuant to the National Environmental Policy Act. In the EU, market authorization is overseen by the European Medicines Agency (EMA), and an EA is required under Directive 2001/83/EC. Both agencies have published guidance documents outlining EA requirements with the EMA issuing an updated draft guidance in late 2018. While the EA requirements of veterinary drugs is largely harmonized, the framework for conducting EAs of human drugs is much less harmonized. The objective of our analysis was to compare and contrast the EA framework in the US and EU. We reviewed current guidance and incorporated recent EA experience. We identified some general similarities such as the prioritization of endocrine active drugs, the use of tiered testing frameworks, and the preference for guideline studies conducted in accordance with GLPs. However, we identified many important differences in the FDA and EMA frameworks including the production and use criteria that trigger EAs, the points at which physicochemical properties are considered, and the specific tests needed to satisfy EA requirements. For example, in the US, the predicted annual production volume may qualify a drug for EA exemption; while in the EU, both the indicated dosage and certain physicochemical properties must be considered for potential EA exemption. Our analysis illustrates the need for careful and early planning by pharmaceutical companies to ensure global EA compliance.

2772 Threshold for Anaphylactoid Reaction to Polysorbate-80 in Canines

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Polysorbate 80 (PS80; Tween 80) functions as a dispersing agent or solubilizer in many pharmaceuticals, and as a stabilizer in biopharmaceuticals. Topical or parenteral administration of the low doses of PS80 in biopharmaceuticals has been associated with mild allergic reactions, including local injection site reactions. High doses of PS80, such as levels found in traditional Chinese medicines, have been linked to systemic effects consistent with anaphylactoid-type reactions, which are characterized by the direct release of histamine from mast cells (degranulation). Nonclinical safety assessments of PS80 *in vivo* have mainly focused on canine model systems, a species well established to be particularly sensitive to PS80. However, there is conflicting data about the dose and route of administration of PS80 required to elicit an anaphylactoid-type reaction in this model system. Therefore, studies in anesthetized and conscious dogs were conducted to better define this threshold dose using multiple dosing regimens (with 0.25% PS80), based on a combination of cardiovascular data, clinical signs, and biomarkers of mast cell degranulation. An IV bolus of 1 mg/kg PS80, a 30-minute IV infusion of 1 mg/kg, or subcutaneous bolus of 20 mg/kg elicited a positive anaphylactoid reaction including increased heart rate, hypotension, and clinical signs associated with anaphylactoid reactions (e.g., reddened muzzle). However, no effects were observed with a subcutaneous injection of PS80 (0.25%) up to 15 mg/kg, IV infusions up to 0.5 mg/kg, or an IV bolus injection up to 0.3 mg/kg. These data show that there is a threshold dose for eliciting an anaphylactoid reaction in the most sensitive species (canine) which varies depending on the route of administration. This will allow for safe dosing of biopharmaceuticals with typically low concentrations of PS80 in patients.

2773 The Process and Challenges of Deriving Exposure-Based Limits for Toxicological Risk Assessment for Components and Impurities Present in Cell Therapy Products with Case Studies

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To ensure the safety of drug products, the components within the formulation must be adequately characterized for potential toxicity. Such components may include product and process-related impurities, degradants, solvents, or excipients. In the case of cellular-based therapies, allogenic materials that are stored and processed may also have increased potential for additional components; such as cytokines, nucleases, growth factors, culture media, buffers, and preservatives. As novel cell-based therapies are developed in the clinic, new formulation components are emerging, the safety of which must be considered. Often, nonclinical and clinical data are included in the evaluation to characterize the safety of a component. However, in the case of cell therapies, nonclinical studies may not have been conducted due to the lack of a relevant animal model for toxicology testing. Therefore, additional data must be identified outside the scope of standard toxicology studies to ensure the level of an excipient or impurity present is acceptable for clinical dosing. A systematic and comprehensive review of primary literature databases, regulatory authority websites, and other industry or government literature sources is necessary to consider critical information such as: 1) physical and chemical properties 2) pharmacokinetic/ADME 3) non-clinical toxicology and immunotoxicology 4) clinical safety data, and 5) relevant regulatory limits and/or guidances. This information is summarized in a risk assessment or toxicology monograph in an attempt to derive a health-based exposure limit, e.g. a Permissible Daily Exposure (PDE), or to ascertain key data gaps that may require additional toxicity testing consistent with regulatory guidance (ICH Q3C). The monograph can then be used to set manufacturing specifications during process development or justify specification limits to a regulatory authority. We present the literature review and risk assessment process, including (when applicable) the derivation of an exposure limit in the context of case studies from cell therapy drug products. Case studies are presented and include cytokines, serum used in media, extraneous cells, and a residual vector. Options for when a PDE cannot be calculated are presented and discussed in terms of the impact on the program.

2774 Gut-Selective Exposure of TD-1473 Limits Systemic Effects of Janus Kinase Inhibition in Nonclinical Species

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TD-1473 is an orally-delivered, gut-selective pan-Janus kinase (JAK) inhibitor under investigation for the treatment of inflammatory bowel disease. Gut selectivity was designed into TD-1473 to maximize exposure in the gastrointestinal tract and limit systemic exposure at therapeutic doses, with the goal of minimizing the known systemic pharmacologic effects of JAK inhibition. This approach was evaluated in repeat-dose general toxicity studies up to 26 and 39 weeks in rats and dogs, respectively, and reproductive toxicity studies in rats and rabbits. In all studies, minimal to no systemic findings were associated with TD-1473 at clinically-relevant plasma exposures. At 1000 mg/kg/day in rats, systemic exposures exceeded the range of cellular JAK IC_{50} values for up to 9 hours and resulted in minimal, non-adverse effects on white and red blood cell parameters and decreased splenic weight and lymphoid cellularity. Similarly in dogs, minimal TD-1473 systemic exposures were achieved at supratherapeutic doses (30 mg/kg/day) and were limited to primary pharmacologic changes in red and white blood cell parameters and decreased hematopoietic and lymphoid cellularity. At higher doses (100 mg/kg/day), chronic administration resulted in systemic exposures exceeding JAK IC_{50} values for up to 24 hours and pharmacologic decreases in immunosurveillance characterized by dermal *Demodex* infections and papillomas secondary to systemic JAK inhibition in dogs. In a rat fertility and early embryonic development toxicity study, there were no changes in mating, fertility, estrous cycling, or early embryonic survival up to the limit dose of 1000 mg/kg/day. In embryo-fetal development toxicity studies, TD-1473 was evaluated up to the limit dose and the maximum maternally tolerated dose (60 mg/kg/day) in pregnant rats and rabbits, respectively. No TD-1473-related changes in fetal evaluations were present in either species, consistent with minimal fetal plasma exposure. When plasma exposures at the no adverse effect level in each study were compared to exposures achieved in a Phase 1 multiple ascending dose study in healthy volunteers, TD-1473 exhibited 9.50- to 41.8-fold ratios at the highest tested clinical dose (300 mg QD). In conclusion, the gut-selective approach exemplified by TD-1473 resulted in minimal to no systemic findings in rats, rabbits, and dogs at therapeutic doses.

2775 Interchangeability of Mainland Southeast Asian Sourced Nonhuman Primates Based on Anatomic and Clinical Pathology Parameters

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As non-human primates (NHP) are frequently used in drug safety assessment, particularly cynomolgus monkeys, the importation of this species represents a constant challenge for the biopharmaceutical industry. The recent increase in demand of cynomolgus monkeys in North America and Europe, combined with the economic downturn of 2009-2011 has led to a significant shortage in NHPs. Recent literature compares the genetic profiles of geographically distinct subpopulations of cynomolgus monkeys and there is a general agreement that the species can be subdivided following ancient differentiation and geographical dispersal in two distinct Asian populations: one continental (Indochina; more specifically Cambodia and Vietnam) and one insular (Philippines, Indonesia and Mauritius). To support the interchangeability between cynomolgus monkeys of Chinese and Indochinese origins, and to improve their availability, the historical control data in anatomic pathology and in-house reference intervals in clinical pathology were compared between cynomolgus monkeys of Cambodian and Chinese origins. Anatomic pathology historical control macroscopic, microscopic and organ weight data were collected from 78 cynomolgus monkeys of each sex of Cambodian and Chinese origin, aged 2.7 to 4.1 yrs, comprising the control groups from 45 toxicity studies evaluated at Charles River Laboratories (CRL), Mattawan, MI, between 2016 and 2019, while clinical pathology data (hematology, serum chemistry, and coagulation) were evaluated from 120 naïve cynomolgus monkeys of each sex of Cambodian and Chinese origin, aged 2.4 to 9.4 yrs, housed at CRL, Reno, NV, in 2013. When available, these control data were also compared to concurrent data reported in recent literature, including control data from Vietnamese cynomolgus monkeys. Based on these comparisons, there were no biologically relevant differences in macroscopic or microscopic changes, organ weight data, or clinical pathology parameters between the subpopulations of cynomolgus macaques from mainland Southeast Asia (China, Cambodia or Vietnam). Furthermore, the anatomic pathology historical control data were all comparable to the common reported incidental findings in cynomolgus monkeys of various origins. These data support the interchangeable use of different subpopulations of cynomolgus monkeys from mainland Southeast Asia in our industry.

2776 A Seahorse HEPATOPAC Model for Integrating Metabolic Activity into Assessments of Mitochondrial Toxicity Potential of Pharmaceuticals

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Mitochondrial dysfunction is broadly considered a contributing factor for drug-induced toxicity *in vivo*. To avoid mitochondrial liabilities, some pharmaceutical firms routinely screen drug candidates for mitochondrial toxicity *in vitro*. However, evaluations have largely relied upon use of cells and models (i.e. HepG2 cells and isolated mitochondria) that are deficient in drug metabolic conversion and allow only for short-term treatment. We have developed the Seahorse HEPATOPAC® model to assess mitochondrial toxicity *in vitro* under conditions where *in vivo*-like drug metabolism capability is present and extended exposures of drug is possible. This platform integrates rat HEPATOPAC® with Seahorse technology that examines mitochondrial respiration and function, in conjunction with metabolomics approaches to inform biochemical pathway perturbations. 25+ model mitochondrial toxicants and drug candidates have been evaluated using the model. We will summarize the findings and present case studies to demonstrate unique value of Seahorse HEPATOPAC® that results in detection of activity absent in metabolically-deficient cells and correlation of *in vitro* and *in vivo* toxicity observation. We will also discuss utility of the model to inform on mitochondrial liability of drug candidates and impact of metabolic transformation on risk assessment.

2777 Adverse Neuroelectrophysiology Changes of a Hepatitis B Virus Antiviral in 13-Week Rat and Cynomolgus Monkey Studies

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The neurotoxicity potential of GS-8873, a small molecule intended for Hepatitis B Virus (HBV) transcript inhibition was evaluated in rats and monkeys in 13-week GLP toxicity studies with 8 weeks of recovery. Wistar Han rats and Cynomolgus monkeys were dosed daily by oral gavage at 2, 6, 20 and 60 mg/kg/day and 0.5, 1.5, 3 and 6 mg/kg/day, respectively. Area-under-the-curve (AUC) exposure margins were 3- to 70-fold for the rat or 4- to 40-fold in monkeys compared to the predicted efficacious clinical dose. Neuroelectrophysiology parameters were assessed during Weeks 4 and 13 of the dosing phase and at 4 and 8 weeks after the last dose administered. Adverse electrophysiology effects were observed in both species at all doses in a dose-dependent manner when compared to age-matched vehicle controls. Changes in nerve impulse latency with decreased amplitude and nerve conduction velocity (NCV) were evident in both rats and monkeys as early as Week 4. Week 4 mean nerve impulse latency values were increased in the rat caudal nerve (5 to 8%) and monkey cauda equina (5 to 10%) across dose groups. Week 13 nerve impulse latency in rats worsened across dose groups to significantly increased values for the caudal (15 to 41%), digital (12 to 29%) and tibial nerves (7 to 18%) with concomitant NCV reductions in the caudal (-12 to -28%) and digital nerve (-9 to -20%). In monkeys at Week 13 of dosing, significant increases in nerve impulse latency values in the cauda equina (18 to 31%) and sural nerve (16 to 24%) were noted with reductions in peroneal nerve NCV (-11 to -23%). Electrophysiology parameters did not return to age-matched vehicle control values after 8 weeks of recovery although some reversal was evident. Specialized neurohistopathology examinations in the rat at Week 13 and at the end of the recovery phase revealed a primary axonopathy in sensory nerves and the spinal cord. No neurohistopathology effects were observed in the monkey after 13 weeks of dosing or at the end of the recovery phase. In conclusion, the adverse dose and time-dependent functional nerve changes in two species, coupled with the need for a safe, chronically administered HBV therapeutic, resulted in the decision to terminate development of GS-8873.

2778 Safety Assessment of Rolapitant in Juvenile Rats Identified a Critical Exposure Window of Rolapitant Susceptibility in Female Reproductive Systems

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Rolapitant is a potent and selective NK1 receptor antagonist approved for the prevention of nausea and vomiting in the delayed phase associated with highly and moderate emetogenic cancer chemotherapy. Definitive juvenile rat toxicology studies were conducted to support the development of rolapitant in the pediatric population. Rolapitant was administered orally to 3 dose groups (12.5, 25, and 50 mg/kg/day) of juvenile Sprague Dawley rats during postnatal day (PND) 7 to 70, followed by a 42-day recovery period. Findings in female reproductive structure and function were observed, including a delay in balanopreputial separation and acceleration of vaginal patency, lower mean numbers of corpora lutea, reduced early embryonic survival at 25 and 50 mg/kg/day; microscopically, an irreversible decrease in endometrial glands was observed at 50 mg/kg/day corresponding to 2.0~7.5 fold of recommended human exposure. Provided significant irreversible reproductive findings were not noted in the 3-month and 6-month repeat dose studies, wherein PND 49-63 old rats were dosed at similar exposure, it was hypothesized that significant irreversible reproductive effects would be the result of rolapitant exposure only during restricted periods of juvenile reproductive development in rats. To delineate the relationship between the exposure window and rolapitant-related reproductive effects, a segment juvenile rat study was conducted in which dosing period was divided in segments to cover defined periods of rat development (PND 7 to 21, 21 to 42, and 42 to 70) corresponding to the age of definite pediatric populations (age 6-month to 2-Y, 2-Y to 12-Y, 12-Y to 16-18-year old in humans, respectively). The dose 50 mg/kg/day was used in all segment-groups to maximize the potential for observation of adverse findings. Rolapitant at 50 mg/kg/day in juvenile rats resulted in adverse effects, including partial or irreversible lower uterine weights correlated with decreased endometrial glands, significant decreases in the numbers of corpora lutea, implantation sites and live embryos and increases in the pre-implantation loss, the numbers of early resorptions and the post implantation loss from PND 7 to 70 and 7 to 21 groups, but not in PND 21 to 42, or PND 42 to 70 groups. These findings were indicative of a critical window of rolapitant susceptibility on female reproductive organs between PND 7 and 21.

2779 Defining the Edge: A Case Study in Expert Review of *In Silico* Hazard Predictions to Identify Activity Cliffs and Improve Chemical Risk Assessment

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New approach methods for chemical safety assessments are seeing increased regulatory acceptance and use across multiple contexts including medical device and pharmaceutical safety, chemical registration, and product stewardship initiatives. Expert review of predictive methods is a critical step in making informed hazard and risk conclusions. Often, these methods rely on data for structurally similar chemicals either directly *via* read-across or indirectly through nearest-neighbor *in silico* models. While chemicals may be similar in molecular weight, relevant functional groups, and chemical character, they may not behave similarly *in vivo*. In this case study, we investigated a test set of structurally similar compounds selected from established databases of local lymph node assay (LLNA) results. All compounds in the test set share a carbonyl moiety, and trigger the "Schiff base formation" alert for sensitization in Toxtree (v 3.1.0). Lastly, the test set was filtered through a binning clustering model to ensure a reasonable degree of quantitative structural similarity (ChemMine similarity cutoff = 0.4). The resulting set consisted of 12 substituted benzenes of similar molecular weight (106 – 190 g/mol), and predicted physiochemical properties relevant to skin sensitization (water solubility > 100 mg/L; K_{ow} range 0-3). The test set represented a range of experimental results, from non-sensitizing to moderately sensitizing, and a false positive hazard prediction rate based on the Schiff base formation alert alone of 25%. Further review of chemical structures suggest the distance of the carbonyl moiety from the benzene ring to be a possible contributing factor with respect to sensitization potential. Nine of the compounds from chemical test set with experimental data for mutagenicity were also evaluated *via in silico* methods in accordance with ICH M7 guidelines. The expert-rule program Toxtree falsely predicted 8 out of 9 compounds to be potential mutagens. In contrast, (Q)SAR program VEGA (v 1.1.5), and expert rule program Derek Nexus (v 2.2) accurately predicted all 9 compounds to be non-mutagenic. This case study demonstrates the importance of expert review when applying predictive toxicology methods, and highlights a possible activity cliff with regards to Schiff base formation and skin sensitization potential.

2780 Evaluation of Metastatic Pathway Inhibition by Novel Ruthenium-Based Metallodrugs Using the Zebrafish Model

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Cancer progression into metastasis is an incredibly complex, multistep process that is the major cause of cancer-related deaths. Targeting this cancer phenotype would result in decreased mortality. However, therapeutic efficacy is difficult to evaluate *in vitro* due to the complex nature of metastasis. The widely accepted model of testing metastasis is immunosuppressed nude mice, but the field agrees there are limitations and drawbacks to this model. Additionally, small quantities of new drugs make statistical power in mice difficult. Although new potentially therapeutic metallodrugs are being synthesized at high rates, there is currently no robust method for evaluation of toxicity or efficacy in mice or other model organisms. In fact, New Anti-Tumor Metastasis Inhibitor (NAMI-A), a ruthenium (Ru)-based complex, showed antimetastatic properties *in vitro* and in the nude mouse model, but failed clinical trials. As such, there is a need for an alternative method of evaluating therapeutic efficacy, to prioritize the most promising of candidate compounds**.** We investigated two novel Ru-based compounds, LCR134 and PMC79, for metallodrug evaluation in zebrafish. A structure-activity relationship between these compounds and NAMI-A, made antimetastatic activity promising. To evaluate antimetastatic potential, we conducted morphometric anti-angiogenic assays, gene expression analysis and wound repair. LCR134 showed no significant fold changes of six genes involved in metastatic progression. However, the majority of these genes were down-regulated after treatment of with PMC79. In addition, angiogenesis is a critical component of anticancer research. New blood vessels formed at tumor sites are required for tumor survival and growth. Blood vessel growth inhibition was measured after treatment with compounds using live-animal imaging. PMC79 exposure demonstrated larger blood vessel lengths at lower doses and significantly less branching at higher doses. Additionally, preliminary data for wound repair indicated that cisplatin and PMC79 exposure resulted in significantly less tissue regeneration and cisplatin caused significant upregulation of proliferating cellular nuclear assay. Together, these zebrafish-based assays could offer an alternative model for assessing anti-metastatic pathways. *Supported by: NJAES-Rutgers NJ01201, NIH-MIEHS P30 ES005022, and Training Grant T32-ES 007148, FCT (project UID/QUI/00100/2013), FCT2013 Initiative project IF/01302/2013 FCT, POPH and FSE - European Social Fund. Ph.D. Grant (SFRH/BD/100515/2014).*

2781 Preclinical Toxicity Evaluation of LUM-001 (Cyclocreatine) in Sprague Dawley Rats

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LUM-001 (cyclocreatine) is being evaluated for its preclinical toxicity in Sprague Dawley rats. Deionized water as a vehicle control article or LUM-001 was administered by oral gavage twice daily (approximately 12 ± 1 hours apart) in rats up to 26 weeks (60 and 200 mg/kg/day group males and females, and 600 mg/kg/day females) followed by a 28-day recovery period. Due to increased incidences of seizures, the 600 mg/kg/day dose group males were dosed for 16-weeks followed by a 14-week recovery period. Seizure incidences were greater at 600 mg/kg, while occasional incidences were also observed at lower doses. In males, there were no test article-related changes in the food consumption or body weights. In females, increased body weight in the 600 mg/kg/day dose group was consistent from Day 22 through Day 162 (5.8-14.3%). During FOB examination, convulsions in open field, a general attenuation of activity levels in the home cage, and lower rectal temperature were observed in males and females at 600 mg/kg/day, males at 200 mg/kg/day, and females at 60 mg/kg/day at Week 13. The changes in clinical pathology that were considered potentially related to the test article administration included decreased serum and urine creatinine, increased serum ALT and AST in both the sexes; increased serum ALP in males only; and decreased urine potassium in females only. Upon histopathological evaluation, higher incidence of vacuoles in brain at 600 mg/kg/day in both sexes were observed. After 14 weeks of recovery, vacuoles in 600 mg/kg/day males were completely recovered but only a partial recovery was observed after a 28-day recovery period. Thyroid follicular atrophy and follicular cell hypertrophy was observed at a higher incidence in 200 and 600 mg/kg/day dose group males, and 600 mg/kg/day dose group females. Moreover, increased incidences of minimal colloid mineralization in thyroid glands were observed in ≥60 mg/kg/day dose group males and females, while some incidences were also observed in recovery control animals. The incidences and/or severity of seminiferous tubular degeneration and /or interstitial edema in testes were increased in a dose dependent manner at ≥200 mg/kg/day doses with a single incidence at 60 mg/kg/day dose. Mean half-life of LUM-001 was between 3.5 to 6.5 hours. *Disclosure: The research was funded under NCI Contract No. HHSN261200800001E.*

2782 Preclinical Safety Evaluation of LUM-001 (Cyclocreatine) in Beagle Dogs

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LUM-001 (cyclocreatine) is being evaluated for preclinical toxicity in beagle dogs. Deionized water (control) or LUM-001 was administered by oral gavage twice daily (approximately 12 hours apart) to dogs at 40, 80, and 150 mg/kg/day doses followed by a recovery period. Due to toxicity, different groups were terminated earlier than the originally planned 39 weeks of dosing. Animals in the 40, 80 and 150 mg/kg/day dose groups completed 160, 106, and 55 days of dosing, respectively, followed by a 30, 55 and 106 days of nontreatment recovery period, respectively. Of the 12 animals (6 males and 6 females)/group, 3 (25%), 7 (58%), and 7 (58%) were euthanized and/or found dead in the 40, 80, and 150 mg/kg/day dose groups, respectively. In general, LUM-001 was absorbed rapidly with group mean T_{max} values typically within 1 hour. On Day 1, the terminal half-life ranged between 2.17 to 2.79 hours; however, on the final day of dosing, it ranged between 5.80 to 8.77 hours for males and between 10.3 to 13.1 hours for females. Moribundity began with inappetence, frequent emesis and stool abnormalities including a lack of stool, diarrhea, or soft and mucoid stool followed by weight loss and lethargy. Death in the 40, 80 and 150 mg/kg/day dose groups was often preceded by acute respiratory distress. Changes in hematological parameters that were considered to be potentially related to test article included decreased lymphocytes, increased monocytes and increased neutrophils which were likely associated with pulmonary inflammation at all dose levels. The changes in clinical chemistry that were considered test article-related included increased ALP, ALT, AST and creatine kinase, as well as decreased total protein and creatinine. Histopathological evaluation revealed adverse findings in the lungs in all dose groups, of congestion, edema, cellular infiltration, fibrin, and/or hemorrhage. This was consistent with gross findings in the lungs as well as acute respiratory distress, which was determined to be the cause of the death in the 40 and 80 mg/kg groups. The specific cause of death in the 150 mg/kg/day group was not known; there was a lower incidence of lung lesions in this group that could be due to the shorter dosing duration and/or longer recovery period. Additionally, animals in all test article dose groups were found to have perinuclear, cytoplasmic vacuolization in the heart, kidneys, skeletal muscle of the biceps femoris and/or tongue, as well as the smooth muscle of the gastrointestinal tract, aorta, and/or urinary bladder. Taken together, a no-observed-adverse-effect level (NOAEL) could not be determined. *Disclosure: The research was funded under NCI Contract No. HHSN261200800001E.*

2783 Safety Assessment of Metarrestin in Dogs: A Clinical Candidate Targeting a Subnuclear Structure Unique to Metastatic Cancer Cells

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Pancreatic cancer is a leading cause of cancer-related deaths. 90% of patients with stage IV disease die within one year of diagnosis due to complications of metastasis. The perinucleolar compartment (PNC) is a subcellular structure whose formation closely associates with metastasis. To support the introduction of Metarrestin, a PNC inhibitor, into clinical trials its toxicity, toxicokinetics and safety pharmacology were evaluated in beagle dogs following every other day oral (capsule) administration for 28 days. Based on the results of an earlier pilot toxicity study, the doses selected were: 0.25, 0.75 and 1.50 mg/kg. The test article dosing formulations were prepared at least once weekly (20 mg/mL) in a vehicle of 80% (v/v) Labrasol® ALF / 20% (v/v) caprylic acid. After oral administration, overall median T_{max} across all doses was 3 hours with no sex differences and no consistent relationship to dose. On Day 27, mean $t_{1/2}$ over 168 hours was estimated to be 55.5 hours. Mean $t_{1/2}$ was similar between females and males and did not vary with doses. There was no sex difference in systemic exposure at any dose level or on days 1 and 27. Metarrestin accumulated from Day 1 to Day 27 at all dose levels and in both sexes by an overall factor of about 2.34. Systemic exposure to metarrestin (as C_{max} and AUC_{last}) increased as dose increased on both Days 1 and 27. No mortality occurred during the dosing period; however, treatment-related clinical signs of toxicity consisting primarily of hypoactivity, shaking/shivering, thinness, irritability, salivation, abnormal gait, tremors, ataxia and intermittent seizures were seen

in both sexes at the 0.75 and 1.50 mg/kg dose levels. Treatment-related effects on body weight and food consumption were seen at the mid and high dose levels. Safety pharmacology study showed no treatment-related effects on blood pressure, heart rate, corrected QT, PR, RR, or QRS intervals, or respiratory function parameters. Based primarily on clinical signs of toxicity, the No Observed Adverse Effect Level (NOAEL) in dogs was considered to be 0.25 mg/kg metarrestin after every other day dosing for 28 days with a mean of male and female C_{max} = 82.5 ng/mL and AUC_{last} = 2521 hr*ng/mL, on Day 27. *Acknowledgments: This work was supported by Leidos Contract No. HHSN261200800001E under BrIDGs/NCATS Program.*

2784 Safety Assessment of Metarrestin in Rats: A Clinical Candidate Targeting a Subnuclear Structure Unique to Metastatic Cancer Cells

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Pancreatic cancer is a leading cause of cancer-related deaths. 90% of patients with stage IV disease die within one year of diagnosis due to complications of metastasis. The perinucleolar compartment (PNC) is a subcellular structure whose formation closely associates with metastatic potential of cancer cells. To support the introduction of Metarrestin, a PNC inhibitor, into clinical trials we evaluated its toxicity and toxicokinetics in rats when administered every other day by oral gavage for 27 days followed by a 28-day recovery period. Based on earlier pilot toxicity finding, this study consisted of four dose groups: vehicle; 7; 20 and 40 mg/kg. The test article dosing formulations were prepared once weekly at concentrations of 1.75, 5, or 10 mg/mL in a vehicle of 80% (v/v) Labrasol® ALF and 20% (v/v) caprylic acid. Metarrestin levels were detected in plasma as early as 30 minutes after dosing; however, absorption was slow as T_{max} ranged from 4 to 8 hours regardless of dose and sex. Systemic exposure was higher in females than in males at all doses but the difference was less than two fold. Metarrestin appeared to accumulate moderately from study days 1 to 27 in both sexes at all dose levels, with accumulation in females being slightly greater than males. Over all dose levels, the ratios of C_{max} and $AUC_{0\text{-}48}$ were 1.23 and 1.44, respectively, for an overall ratio of 1.33. No treatment-related deaths were seen. No treatment-related adverse signs were noted during the daily cage-side clinical observations or the weekly detailed physical examinations. No treatment-related or toxicologically significant findings were noted for body weights, body weight gains, food consumption, ophthalmic examinations, functional observational battery (FOB), clinical pathology, organ weights, and gross and microscopic pathology. Thus, due to the lack of treatment related toxicologically significant findings, the No Observed Adverse Effect Level (NOAEL) for this study was 40 mg/kg dose administered every other day for 27 days with a mean of male and female C_{max} = 2,141 ng/ml and AUC_{last} = 78,594 hr*ng/mL, on Day 27. *Acknowledgments: This work was supported by Leidos Contract No. HHSN261200800001E under BrIDGs/NCATS Program.*

2785 Comparison of Historical Control Data in CD-1 Mice When Single- versus Group-Housed and Considerations for Successful Group-Housing of Male Mice

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Male and female mice on toxicology studies have historically been single-housed in stainless steel wire mesh-bottom cages. Subsequently female mice were group-housed in stainless steel solid bottom cages with bedding or in polycarbonate bins with bedding whilst male mice were single-housed due to behavior challenges. Based on continued efforts, male mice have been successfully group-housed for the duration of studies out to two-years in similar housing environments in recent years. A number of factors led to the successful group-housing of male mice. One key factor was the age of the mice (pre-puberty) at which male mice were placed together, housing up to at most 3 male mice per cage unit. In addition, providing nesting material and subsequent transfer of a portion of the bedding to the new cage during cage-exchanges, the order of manipulation (males before females or changing gloves if handling females before males), and minimizing disruptions during the day have all been aspects contributing to the successful outcome. Comparative data from single-housed *vs* group-housed mice were reviewed to assess the effect housing conditions have on body weight, food consumption, survival or palpable masses. Mean body weight for group housed mice appears higher when compared to single-housed mice whereas the opposite holds true for food consumption where group housed mice appear to consume less than single housed mice. Incidences of survival and palpable masses were comparable between the single- and group-housed mice. In conclusion, although challenging, male mice have been successfully group-housed in studies conducted in our facility. An apparent effect on both body-weight (higher) and food consumption (lower) have been noted and should be considered when doing any comparisons of historical data.

2786 Bioluminescent Assays for Investigating Insulin Action and Steatosis

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Metabolic syndrome is a cluster of conditions - including high blood pressure, central obesity, elevated blood sugar, and high levels of lipids. Two factors connecting these conditions are the action of insulin and the accumulation of lipids (i.e. steatosis). To study these processes, we have developed a set of bioluminescent detection assays for a variety of metabolites (e.g. glucose, lactose, glutamine, glutamate, glycerol, triglyceride, cholesterol, and cholesterol esters). These assays use the same core technology that couples specific metabolite dehydrogenases to the production of NAD(P)H and the generation of light. We have used these assays to follow the differentiation and functional characterization of 3T3L1-MBX adipocytes. To follow differentiation, we used a triglyceride detection assay that does not require organic extraction yet provides quantitative detection of intracellular triglyceride accumulation, from 10 µM in undifferentiated fibroblasts to 590 µM in mature adipocytes. The mature adipocytes were further characterized by measuring the insulin effect on two major insulin regulated pathways, glucose uptake and lipolysis. Insulin dose response curves showed a 10-fold increase in glucose uptake with an IC50 of 0.1 nM, and this was inhibited 2-fold by 50 µM LY294002, a PI3K inhibitor. These mature adipocytes also show a 4-fold increase in extracellular glycerol release upon stimulation with 25 nM isoproterenol, a lipolysis inducer, and this was suppressed 2-fold by 150 nM insulin. We have also used these metabolite detection assays to study liver models. By measuring extracellular glucose, we measure a 4-fold inhibition of gluconeogenesis in liver microtissues by 10 nM insulin. Moreover, using HepG2 cells as a model for nonalcoholic fatty liver disease (NAFLD), we measure a 5-fold increase in intracellular triglyceride upon overnight incubation with 0.3 mM BSA-bound fatty acids. For measuring insulin and glucagon, we have developed a simple "add-and-read" bioluminescent immunoassay. This technology is a structural complementation system comprised of an 18 kD protein and an 11 amino acid peptide that when combined form an active luciferase. By attaching these subunits to antibodies, we have developed in-solution, no-wash immunoassays for measuring insulin and glucagon. The assays are sensitive, with detection limits of 5 -10 pM antigen. Using the insulin immunoassays, we measured a 3-fold increase in glucose-stimulated insulin secretion from INS-1 beta cells.

2787 Taking Toxicology to the Next Dimension: Evaluating the Toxicological Screening Utility of 3D Spheroid Primary Human Hepatocyte Cultures

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Three-dimensional (3D) cell culture models are transforming *in vitro* toxicology through their enhanced translational relevance in exhibiting tissue functionality. Primary human hepatocytes (PHHs) are considered the gold standard for assessing hepatic responses to xenobiotics *in vitro*. However, their toxicological screening utility in conventional 2D culture models is limited due to their short culture longevity (days), the limited number of cells from individual donor livers, the high degree of variability between donor preparations, and high costs. Our goal was to assess the feasibility and human relevance of PHHs cultured as 3D spheroids in modeling hepatotoxicity and compare to 3D spheroids cultures of HepaRG, a liver progenitor cell line. Single-donor spheroids (2000 cell spheroids, 384-well plates, 1 spheroid/well) from 3 individual donor PHH preparations were generated in ultra-low attachment plates and their liver functionality was assessed over time. Spheroids from all donor preparations were viable through at least 28 days and maintained physiologically-relevant activities of major drug metabolizing liver enzymes, including CYP3A4, that were equal to or higher than 3D HepaRG at 14 days. 3D PHHs were exposed in 10-point dose response to a set of reference chemicals for 96 hours and assessed for cytotoxicity (ATP and LDH leakage). Troglitazone, a drug withdrawn from the market due to liver injury, more potently caused cytotoxicity in 3D PHHs compared to its safer analogue, rosiglitazone. Aflatoxin B1, a known hepatotoxicant attributed to metabolic activation, caused concentration-related cell death in 3D PHHs at significantly lower concentrations than 2D PHHs and 3D HepaRG, consistent with their enhanced degree of metabolic competence. 3D PHHs were more sensitive to cell death by the withdrawn drug cerivastatin than the safer alternative atorvastatin, and overall more sensitive to both compounds than 3D HepaRG.

The increased sensitivity of 3D PHH over 3D HepaRG may indicate increased hepatocyte functionality and translational utility of primary cells. Future work will use high-throughput transcriptomics to mechanistically evaluate the relevance of biological responses to a diverse set of chemicals across donor preparations. This work demonstrates the ability of 3D PHHs to predict liver injury potential and sets the stage for modeling genetic diversity *in vitro* for toxicological screening.

2788 A New Medium-Throughput Screening Assay for Identifying Chemical-Dependent Changes in ABC Efflux Transporter Activity at the Blood-Brain Barrier

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The blood-brain barrier (BBB) regulates the entry of blood-borne substances to the central nervous system (CNS). ATP-Binding Cassette (ABC) efflux transporters localized to the luminal membrane of capillary endothelial cells are the primary chemical mediators of the BBB and limit transcellular movement of endobiotics and xenobiotics into the brain. Present methods for analyzing transporter activity are hindered by resource and time requirements, limiting their utility for medium- to high-throughput screening. Given the BBB's role in CNS protection and its role as an obstacle to the delivery of CNS-acting drugs, a new method for screening BBB transporter activity is needed. Herein, we describe the development and feasibility of using the ImageXpress® Micro Confocal System (IXM) from Molecular Devices to screen environmental contaminants for their ability to modulate transporter activity in rat brain capillaries. This method uses confocal microscopy to quantify capillary luminal fluorescence of transporter-specific fluorescent substrates at steady state, measuring transporter activity. Freshly isolated capillaries were loaded in a 96-well plate and pre-treated with a series of compounds followed by the application of transporter-specific fluorescent substrates. Capillaries were scanned on the IXM to capture dozens of capillaries per treatment group. Next, capillaries were analyzed using MetaXpress, an adaptable computer program. The analysis program was written to identify capillaries, quantify luminal fluorescence, and exclude non-capillary fluorescence. Presently, we have demonstrated the ability to distinguish controlled and inhibited capillaries and are beginning to identify positive control treatment groups. As a proof of concept, we confirmed that 2,4,6-Tribromophenol significantly reduces P-glycoprotein activity after five hours exposure at 100nM. Once established, this automated screening assay will aid in the rapid identification of toxicants, drug candidates, and other xenobiotic compounds that significantly alter transporter activity at the blood-brain barrier with further implications for therapeutic treatments at other barrier tissues. *This research was supported in part by the Intramural Research Program of NIH/NCI [Project ZIA BC 011476].*

2789 A Microfluidic Thyroid-Liver Platform to Investigate Thyroid Toxicity Mechanisms in Humans and Rats

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The assessment of the human relevance of toxicities observed in animal assays still represents a major challenge for various areas of toxicology. Determining the species-specificity of thyroid effects is key for the regulatory acceptance of the risk assessment. Rodents, which are widely used for regulatory toxicity testing, are particularly sensitive to perturbations of thyroid homeostasis. A recurring problem is thyroid toxicity and determining whether this toxicity is caused by a direct effect or if it is mediated via liver enzyme induction. Emerging microfluidic microphysiological systems (MPS) present a promising approach to reproduce the physiological and toxicological interactions of target organs which up to now can only be investigated in animal studies or during clinical trials. So far MPS technologies have only rarely been adopted by the pharmaceutical or crop science industries due to lack of qualified assays to predict the safety of novel drug candidates and agrochemicals. Here, we present the interconnection of three-dimensional (3D) organ models representing both target organs liver and thyroid of both human and rat origin in a commercially available multi-organ-chip platform. Their systemic functional 14-day homeostasis could reproducibly be demonstrated applying a common culture medium for the first time. Steady glucose consumption, low LDH release and immunohistology of 3D thyroid follicles as well as liver spheroids revealed viability. The organ-specific functions, albumin secretion and urea synthesis for the liver as well as T4 release for the thyroid follicles, were maintained. Finally, a chemically-induced increase in T4 catabolism, determined by accelerated thyroid hormone glucuronidation, was observed when liver spheroids were incubated with reference inducers like beta-naphthoflavone. Thus, we show for the first time a model of the hepatic-thyroid axis within a single *in vitro* assay for two different species. These two chip-based models represent a major step forward to better assess the potential species similarities/differences of toxicity findings observed in rodents with a significant contribution to the 3R principles.

2790 High-Throughput Co-culture as an Emerging Technology for Increased Predictability in Drug and Chemical Screening

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We have worked to established a high-throughput screening (HTS) compatible *in vitro* nephrotoxicity assay complete with conferred hepatocyte bioactivation. Our goal was to expand the traditional nephrotoxicity assay by adding metabolic competence to increase the *in vivo* predictability of these assays while at the same time constraining compatibility to existing HTS infrastructure. Renal cell lines (HEK293's and RPTEC's) were co-cultured with human hepatocytes (HepG2, HepaRG and PHH). Co-culture was performed in 96, 384 and 1536-well microDUO plates (48, 192 and 786 co-culture pairs respectively) to demonstrate scalability. In addition, 96-well microDUO was directly compared to Transwell® plate co-culture to demonstrate the competitive advantage of the microDUO co-culture technology. The microDUO plate enables co-culture in technically simple and HTS compatible manner that seamlessly integrates with existing robotic infrastructure. To illustrate HTS compatibility we utilized a range of HTS equipment to complete the assays. The microDUO wells were coated with 15ug/ml fibronectin and 0.5mg/ml Collagen Type 1 (HepaRG and PHH wells only) using a Thermo Multidrop™ Combi dispenser. After coating, the plates were washed using a BlueCatBio plate washer. Cells were seeded at 3,000 and 12,000 cell per well for kidney cells and hepatocytes respectively using the Multidrop™ Combi dispenser. Chemicals were dosed using a Labcyte Echo droplet dispenser and cell viability was quantified using CellTiter-Fluor and CellTiter-Glo on a BMG CLARIOstar and PHERAstar plate reader. Renal and hepatocytes cell lines were exposed to 10 different concentrations ranging from 0.1uM to 100uM (6 different concentration in 96-well microDUO) of known nephrotoxic compounds including Sunitinib Malate, Aflatoxin B1, Cyclophosphamide, Aristolochic Acid I, Arsenic Trioxide, Cadmium chloride, and Potassium Dichromate. We have observed that compounds like Sunitinib Malate, Aristolochic Acid I, Cyclophosphamide and Cadmium Chloride, showed cytotoxicity in monoculture but detoxification in co-culture with hepatocytes. On the other hand, Aflatoxin B1 had little to none cytotoxic effect in monoculture but cytotoxicity was observed in co-culture with hepatocytes. Future directions include generating assays to study pulmonary toxicity, digestive track toxicity, neurotoxicity and cardiotoxicity. A fully metabolically competent and truly HTS compatible toxicity assay would be invaluable in identifying potentially toxic chemicals in preclinical development and toxicity testing.

2791 Worm-on-a-Chip Technology: An Emerging 3R Model in Toxicity Testing

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The nematode *Caenorhabditis elegans* is an excellent model organism when considering alternative approaches in line with the 3R principles. These tiny worms (1-mm long) have been widely used for studying development, stress, and aging and, more recently, as a new model in environmental toxicology. Genes and signaling pathways are well conserved between *C. elegans* and humans, and the development map of *C. elegans* is well described, with >40 years of genetic studies. In this study, we took advantage of a microfluidic system combining high-resolution imaging and image analysis algorithms developed by Nagi Bioscience to evaluate the suitability of this *in vivo* model for assessing the potential toxicity of a battery of known compounds in a high-throughput manner. Hermaphrodite worms were first confined in dedicated microfluidic chambers (1-3 worms per chamber) at larval stage L1. Ten to twenty worms were exposed continuously per condition and experimental repetition to ten chemicals (such as lithium chloride, bisphenol A, and acrylamide) at a wide range of concentrations. The worms were monitored every hour for a 130-h longitudinal observation at constant temperature. Automated image processing and multi-phenotypic analysis were then performed to determine the specific toxicity profile of each testing chemical. We first observed that inter-individual variability was improved by synchronizing worms at the same developmental stage by starvation prior to exposure to the testing chemicals. The no-observed -adverse -effect -level was determined for each testing chemical. Various phenotypic readouts were extracted during the assay (such as worm size, worm lethality, and worm reproduction), allowing ranking of the compounds on the basis of severity of their adverse

effects relative to the respective control. This study highlights the multiple advantages of this new high-throughput screening approach, including: (i) good reproducibility and accurate results by using standardized protocols, (ii) precise and dynamic dosing of compounds with low liquid consumption, and (iii) phenotypic readouts in real-time and at single-animal resolution. Combining a microfluidic system with a high-content screening method makes *C. elegans* a promising model for next-generation toxicity testing.

2792 Application of Cryopreserved Human Intestinal Mucosa (CHIM) in the Evaluation of Regional Differences in NSAID Enterotoxicity

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NSAID-associated enterotoxicity is a well-recognized adverse effect of this class of mediation that are widely used for the alleviation of pain and inflammation. NSAID is known to cause serious mucosal damage, resulting in perforation and bleeding throughout the gastrointestinal tract which may have fatal outcomes. Early identification and elimination of drug candidates with serious enterotoxicity therefore will aid the development of drugs acceptable enteric safety. We report here the development of an *in vitro* assay for the evaluation of NSAID-related human enterotoxicity using a novel *in vitro* experimental model of the human small intestine, namely, the cryopreserved human intestinal mucosa (CHIM). We have recently reported preparation of CHIM from human small intestines from organs procured but not used for transplantation. CHIM were found to retained high viability and drug metabolizing enzyme activities upon recovery from cryopreservation. We report here results of our investigation on the application of CHIM to evaluate potential regional differences in NSAID-induced enterotoxicity. In this study, CHIM were isolated from 10 12-inch segment of the small intestines. Robust drug metabolizing enzyme activities were observed, especially for CYP2C9 (diclofenac 4-hydroxylation), CYP3A4 (midazolam 1-hydroxylation and testosterone 6b-hydroxylation), and UGT (7-OH-coumarin glucuronidation). Using the 10 12-inch regions from the small intestines of three donors, the enterotoxicity of two NSAIDs: acetaminophen and naproxen, were evaluated. Dose-dependent decrease in viability quantified as cellular ATP contents were observed for all regions. Naproxen was found to have a higher *in vitro* enterotoxicity in CHIM for in most CHIM segments from all three donors, a result consistent with reported clinical findings. The resulting EC50 values of acetaminophen and naproxen were within the drug concentrations in the intestinal lumen upon oral ingestion. Our results suggest that CHIM may represent a useful model for the rank ordering of drug candidates for drug development based on *in vitro* enterotoxicity. CHIM can also be applied towards the understanding of the mechanism of enterotoxicity, especially in the correlation between enteric drug metabolism and enterotoxic potential. The individual and regional differences in NSAID enterotoxicity suggest genetic and environmental factors may play a role in the manifestation of enterotoxicity.

2793 *In Vitro* Cell-Based Cytotoxicity and T Cell Activation Assays to Assess Safety and Efficacy of Engineered T Cell Therapies

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Chimeric antigen receptor (CAR) and T cell receptor (TCR) engineered T cells are part of a big wave of immunotherapies showing great promise in cancer clinical trials. With the first T cell based therapies targeting hematological malignances now approved, their next challenge is solid tumors. Solid tumors create an additional challenge due to the lack of tumor specific target antigens, posing significant safety risks, i.e. on-target on-tumor, on-target off-tumor and off-target toxicities. Toxic effects previously reported vary from mild-severe cytokine release syndrome (CRS) to neurotoxicity and death. We have developed *in vitro* assays utilizing primary human cells from healthy tissue and/or differentiated iPCS-derived cells to assess on-target off-tumor and/or off-target cytotoxicity for engineered T cell therapies. The presence of CAR-T cell mediated cytotoxicity was measured through co-culture with healthy human primary cells to assess unwanted CAR-T reactivity as well as a high target antigen expressing control cancer cell line to confirm CAR-T functionality. The human primary cell type was selected based on its potential safety risk by establishing low level protein expression of the target antigen. Readouts included IFNγ production determined by MesoScale Discovery platform as a measure of T cell activation and Hoechst/PI staining of target cells by flow cytometry. Our study generated high quality data of the CAR-T cell, confirming functionality by showing consistent T cell activation and killing against a positive control cell line. Moreover, we were able establish a clear absence of activity against the primary human cells thus providing insight into the safety of the CAR-T therapy. The developed safety assays provide a robust and rapid platform to assess on-target off-tumor and off-target effects within immuno-oncology therapies, either TCR or CAR-T cells, in both early stage development or late stage testing of the therapeutic product. Through inclusion of a wide range of human primary cells, both high risk tissues and major organs at risk of off-target toxicity, a clear safety profile can be generate *in vitro* for these novel T cell therapies. Safety risks associated with cell based IO-therapies is the biggest challenge for the success of these therapies, performing a thorough safety screen on healthy primary human tissues is therefore crucial.

2794 Vitrofluid: A Versatile Multi Organs-on-a-Chip Platform

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In vitro models have limitations in mimicking the processes occurring in the human body. Their inability to mimic complex physiological processes contributes significantly to failure. A combination of 3D *in vitro* models with an engineered microenvironment has been recently developed. These systems, known as "organ-on-a-chip," have the potential to make drug development processes more effective. Recently, Philip Morris International has created a lung-liver-on-a-chip platform to better assess the toxicity of aerosols. The system is composed of a chip plate with 3 circuits, each composed of 2 connected wells. Each circuit is connected to a pump, ensuring medium circulation. In this study, we tested the performance of air-liquid interface bronchial epithelial cultures and HepaRG liver spheroids that were maintained together in the platform for 28 days. The bronchial cultures showed stable transepithelial electrical resistance (TEER) and cilia beating frequency throughout the study. The liver spheroids secreted albumin and maintained their metabolic capacity over the study period. We further tested the role of the liver spheroids in modulating the toxic effects of a known pulmonary toxicant, aflatoxin B1. The results showed that bronchial cultures exposed to aflatoxin B1 in the absence of the liver spheroids exhibited a severe decrease in TEER and adenosine triphosphate content. Conversely, in the presence of the liver spheroids, bronchial cultures were unaffected by the compound. Furthermore, using this platform, we tested whether air-liquid bronchial cultures could be developed faster and more optimally. For this experiment, bronchial cultures were transferred immediately after air-lift and exposed to continuous medium flow for 4 weeks. The cultures were evaluated every week for morphology and functionality. We found that constant medium recirculation improved the homogeneity, pseudostratification, ciliation, and cell polarization of the cultures relative to the cultures matured in standard plates. Collectively, these results demonstrated the versatility of this chip platform and its potential for toxicological evaluation.

2795 Application of Pathway-Specific Permeabilized Human Hepatocytes (MetMax Human Hepatocytes) in the Identification of Activation and Detoxification Pathways

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We have previously proposed that drugs with idiosyncratic toxicity may be identified as drugs where individuals may be sensitized via genetic and environmental factors. Drugs which are metabolically activated and detoxified are hypothesized to have idiosyncratic drug toxicity, as a patient may be sensitized to these drugs if genetic and environmental factors would enhance activation and compromised detoxification. We have recently developed a novel experimental system, cofactor supplemented permeabilized cryopreserved human hepatocytes (MetMax™ Cryopreserved Human Hepatocytes, MMHH). MMHH as an experimental system has the complete phase I oxidation and phase II conjugation drug metabolizing enzyme pathways as that for intact hepatocytes, and the convenience of liver microsomes including storage at -80 deg. and use directly upon thawing without a need for centrifugation and microscopic examination. Further, MMHH can be used at toxic drug concentrations that would diminish drug metabolizing enzyme activities in intact hepatocytes, and that one can select drug metabolizing enzyme pathways via the specification of cofactors. We report here the use of pathway-specific MMHH via supplementation with individual cofactors to identify key detoxification pathways. In this study, cytotoxicity of two protoxicants that require metabolic activation, acetaminophen and cyclophosphamide in HEK293 cells using pathway-specific MMHH as an exogenous metabolic activating system. The pathway-specific MMHH systems were: 1. Oxidation only (MMHH-OX); 2. Oxidation and glucuronidation (MMHH-UGT); 3. Oxidation and sulfation (MMHH-SULT); 4. Oxidation and acetylation (MMHH-NAT), 5. Oxidation and methylation (MMHH-MT); and 6. Oxidation and glutathione conjugation (MMHH-GST). The results showed that the cytotoxicity of both acetaminophen and cyclophosphamide was enhanced in the presence of MMHH-OX,

confirmation metabolic activation of these two protoxicants to cytotoxic metabolites. Cytotoxicity was diminished for acetaminophen in MMHH-UGT, MMHH-SULT, and MMHH-GST, suggesting that all three pathways were important its detoxification. For cyclophosphamide, diminished cytotoxicity was only observed for MMHH-GST, suggesting that GSH-conjugation is the key detoxifying pathway. Our results suggest that pathway-specific MMHH can be used as a tool to aid the identification of key drug metabolic enzyme pathways for metabolic activation and detoxification, leading to the identification of at risk populations and the identification of drugs with idiosyncratic drug toxicity.

2796 *In Vitro* Skin Sensitization Assays: Practical Experiences Performing the DPRA, KeratinoSens, and h-CLAT

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Validation of New Alternative Methods (NAMs) is one of the crucial challenges facing the drive towards animal-free testing today. Much time and effort has been put into generating documents to guide and support validation processes by organisations such as the Organisation for Economic Co-operation and Development (OECD), the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM), the Environmental Protection Agency (EPA) and the Health and Environmental Sciences Institute (HESI) amongst others. To date, formal validation of new assays has required the developer to assess intra-laboratory variability and subsequently inter-laboratory variability to ensure the Final Protocol / Standard Operating Procedure is transferable and the data generated are reproducible. However, the advent of New Approach Methods (NAMs) has introduced complex and specialist instrumentation into assays in the drive to move away from *in vivo* testing and better predict human responses. The consequence of this has been the need for expert knowledge in those techniques in the naïve laboratory adopting the assay and for a more effective transfer of knowledge from the developers to facilitate successful implementation. As a Contract Research Organisation (CRO), we are alert to the challenges of implementing NAMs and have successfully validated a number of these in recent years. Using our experiences with the three *in vitro* skin sensitisation assays (Direct Peptide Reactivity Assay [DPRA], KeratinoSens™ and human Cell Line Activation Test [h-CLAT]) as a case study, we highlight and discuss issues we overcame, questions that have arisen during routine testing for a global client base covering a broad spectrum of substances, and draw conclusions based on our experience for consideration in future documentation.

2797 The Use of Multi-Species Intestinal Organoids as a Preclinical Screen to Assess Gastrointestinal Toxicity

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The mouse small intestinal *in vitro* organoid model (Sato *et al*. in 2009), has been used as a tool assay for several diseases and treatments, including cancer. Gastrointestinal (GI) toxicity is a common and often severe dose limiting side effect of chemotherapy. Symptoms include diarrhoea, dehydration and ulceration which increase susceptibility to infection, partly due to damage of crypt and/or villus structures in the small intestine, impairing the barrier function. As novel targeted therapies are emerging, assessment of their potential GI toxicity remains crucial. We have further developed and validated the organoid model as a screening tool to predict GI toxicity and subsequent mucosal regeneration in four species: mouse, rat, canine and human. The culture conditions have been designed to mimic the stem cell niche and allow cell differentiation and proliferation to occur. All intestinal lineages were present in the organoids derived from each species and the epithelial hierarchy closely resembled that observed *in vivo*. The effect of several chemotherapeutic agents known to induce GI toxicity has been assessed, such as Oxaliplatin, 5-Fluorouracil (5-FU), CPT-11 and its metabolite SN38 and various Tyrosine Kinase inhibitors (TKIs). To assess the effect of the treatment, the number of dead, viable unbranched and branched organoids were counted and IC50 values were calculated and compared between species to inform on species-related sensitivity. MOA data generated by RNA-seq is presented elsewhere in the abstract by Tudor *et al*. The *in vivo* translatability of these models was also assessed. For example, rats have demonstrated that they are more sensitive than mice to 5-FU, experiencing more severe diarrhoea and earlier mortality. The 5-FU IC50 value obtained through the *in vitro* organoid assay supported this observation and was lower for the rat organoids than mouse. Human organoids were more sensitive to CPT-11 than 5-FU which also supports clinical data. Oxaliplatin was the least toxic compound within the human organoid assay, reflecting clinical data that shows the lowest incidence of severe diarrhoea. We conclude that organoids are a predictive preclinical model that can be used to identify potential on-target, off-tissue GI toxicities induced by novel therapeutics. Toxicity and mechanism of action (MOA) can all be addressed *in vitro* to potentially reduce *in vivo* experimentation.

2798 Deciphering Intestinal Toxicity via the Use of Short-Term *In Vivo* Studies and Multispecies Organoid Models Combined with Next Generation Sequencing

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Intestinal toxicity is often manifest as early diarrhea (hours to days post dosing) with histopathology typically examined after a 14 or 28d toxicity study, i.e. long after the onset of toxicity. The epithelial pathologies are often described as villus blunting, crypt atrophy/erosion and hyper/hypoplasia, along with underlying inflammation, hemorrhaging etc. However, given that the epithelium is replaced weekly, with just Paneth and stem cells persisting, the original target cells may be long gone, with new target populations being generated during drug exposure. Deciphering the cause of the toxicity can therefore be a problem, especially when tissues are only collected at the study end. This is compounded by the ever present question of whether toxicity observed in the animal model is representative of the human response. Multiple drug administrations can extend toxicity to additional cell populations depending on timing, bioavailability and status of the cells following the prior exposure. For example, a drug killing the rapidly proliferating epithelial cell population can trigger a regenerative response in more quiescent progenitor cells, making them sensitive to subsequent doses. We have tracked the first cells responding to a single drug administration and used this to predict long term consequences and benchmark new drugs with similar target profiles. For example, doxorubicin has a different target cell profile to 5-FU (clonogenic cells vs the rapidly proliferating cell population respectively). Various Wnt and tyrosine kinase inhibitors have also been profiled. Characterization of gene expression pathways linked to these differing responses were also defined using Illumina Array and HiSeq Next Generation Sequencing platforms. These responses were also evaluated using intestinal organoids derived from 4 key species (mouse, rat, dog and human - described in the abstract submitted by Ubertini *et al*). Using such models we have shown that the responses elicited *in vitro* correlate with *in vivo* data, and that gene expression data for the epithelial responses were also comparable. The use of organoid models therefore allows a faster, cheaper method of evaluating drug toxicity and MOA, deprioritizing drugs with off target cross-reactivities responsible for toxicity (enabling drugs to 'fail faster'). Organoids can also guide the selection of the *in vivo* animal model most closely aligned to the human response.

2799 Optimization of 3D Liver Spheroid Formation and Functionality

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Spheroids have proven to be an extremely useful *in vitro* model in ADME/Tox research. There is a need for more realistic hepatic models such as spheroids, due to the large amount of costly drug candidate withdraws. Spheroid culture conditions are tested in spheroids of individual hepatocyte donors and non-parenchymal cells in order to determine optimal conditions for the highest functionality. Spheroid formation of hepatocytes and non-parenchymal cells will be studied in culture medium supplemented with minimal FBS, seeded at 1 - 1,500 cells per well in a 96-well ultra-low attachment plate. In this study, 3D spheroids were created using Novabiosis cryopreserved primary human hepatocytes and hepatic non-parenchymal cells from individual donors. Here, various seeding densities and compositions of culture mediums are tested, with differing proportions of FBS and other common culture medium additives such as dexamethasone, glutamax, pen/strep, and insulin, as well as varying ratios of hepatocytes:NPCs. Functionality of the Kupffer cell lots used in culture was determined prior to their addition to the culture by the treatment of LPS and measurement of IL-6 and TNF-α production. Functionality of the hepatocytes in the spheroids was measured by ATP production, albumin secretion, and CYP1A2 (Omeprazole 50μM; SigmaAldrich O104), 3A4 (Rifampicin 10μM; SigmaAldrich R3501), and 2B6 (Phenobarbital 1mM; SigmaAldrich P1636) expression. The ATP production was determined using the Luminescent ATP Detection Assay Kit (abcam ab113849). Albumin secretion was measured using the Human Albumin ELISA Kit (abcam ab108788). Hepatocyte spheroid cultures were also characterized for their transporter activity for transporters OCT, BSEP, NCTP, and OATP. Spheroids were also fixed and had H&E staining performed to show morphology. All

spheroid formation was achieved utilizing ultra-low attachment plates (Corning™ 3474). An optimized spheroid model that improves predictability for translational responses to prototypical and novel drug compounds will be presented.

2800 Optimization of Non-parenchymal and Hepatocyte Co-culture Model

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When a drug compound encounters the liver and is metabolized, more than just hepatocytes can play a role. The liver is made up mainly of hepatocytes, but also contain non-parenchymal cells such as Kupffer cells, stellate cells, sinusoidal endothelial cells, cholangiocytes, and a few other cell types. The combination of all the cell types beyond just hepatocytes in culture can provide a very useful *in vitro* research tool in the ADME/Tox field. The co-culture composed of hepatocytes and non-parenchymal hepatic cells create a more realistic liver environment as opposed to a hepatocyte culture alone. Novabiosis explored the co-culture conditions with the goal of optimizing the co-culture to give the longest lasting culture with the highest functionality. Varying ratios of non-parenchymal cells to hepatocytes were investigated. While it is hard to predict the exact cell composition in the liver, it has been estimated that Kupffer cells make up a majority of the non-parenchymal cells, followed in quantity by sinusoidal endothelial cells, immune cells, biliary cells such as cholangiocytes, and finally Stellate cells. It was of interest to explore if these percentages were truly preferred by hepatocytes in cell culture or if the exact composition did not contribute to functionality. The hepatocytes were first plated and cultured for 24 hours before the non-parenchymal cell types were added. Cultures were maintained out to at least 14 days. Functionality of the Kupffer cell lots used in culture was determined prior to their addition to the culture by the treatment of LPS and measurement of IL-6 and TNF-α production. Functionality of the hepatocytes was measured by ATP production, albumin secretion, and CYP1A2 (Omeprazole 50μM; SigmaAldrich O104), 3A4 (Rifampicin 10μM; SigmaAldrich R3501), and 2B6 (Phenobarbital 1mM; SigmaAldrich P1636) expression. The ATP production was determined using the Luminescent ATP Detection Assay Kit (abcam ab113849). Albumin secretion was measured using the Human Albumin ELISA Kit (abcam ab108788). An optimized co-culture model could potentially aid in the prediction of DILI side effects from novel drug compounds.

2802 Processed Clay Can Bind Polychlorinated Biphenyls and Mixtures with High Affinity

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Polychlorinated biphenyls (PCBs) have been detected as prevalent environmental contaminants in water, food and biota. Its accumulation in the fatty tissues of fish and other animals can be magnified during events such as hurricanes, floods, heavy rain, and storms, when PCB contaminated sediments are mobilized and redistributed, thus enhancing exposures and adverse health impacts in vulnerable humans and animal populations at the site of disasters. To address this problem, we have developed a broad-acting and highly effective sorbent for PCBs using montmorillonite clays reported to be safe for consumption in animals and humans. In this study, montmorillonite clays were processed with acid (APMs) and the sorption of six PCB congeners (PCB 77, 126, 153, 157, 154 and 155) on the surfaces of APMs were characterized. To confirm the safety and predict the *in vivo* efficacy of APMs, we used a living organism (*Hydra vulgaris*) that is sensitive to toxins. In a novel application of this work, APMs were included in algae-based feed for commercial oysters (*Crassostrea virginica)* as prophylaxis and treatment against PCB contaminants during disasters and high level exposures. APMs significantly protected hydra against the toxicity of PCBs and common mixtures (Aroclors 1254 and 1260), and reduced PCB uptake and accumulation in oysters when included in the diet. This finding was supported by *in vitro* studies showing tight binding; high capacity, affinity, and enthalpy; and a low therapeutic dose. APMs have been shown to bind a wide range of PCBs and other environmental chemicals, and can be delivered in food, drinking water and animal feed to mitigate toxin exposures in vulnerable humans and animals at the site of disasters (P42 ES027704).

2803 Endocrine Disrupting Chemical Exposure during Pregnancy and Metabolic Reprogramming

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Pregnancy is a critical window of susceptibility to disease, as a woman's body undergoes numerous changes coordinated by hormones. Pregnancy is also characterized by exposures to multiple chemical classes of endocrine disrupting chemicals (EDCs). Recent epidemiological research indicates women exposed to mixtures of EDCs, i.e. phthalates, perfluoronated compounds, bisphenols, etc., during pregnancy have a higher chance of developing Type 2 diabetes in their lifetime. To address the possibility, mouse dams were exposed to relatively low doses of four EDCs that share consequences on metabolic function: Atrazine (ATR - 10mg/kg), Perfluorooctanoic acid (PFOA - 0.1 mg/kg), Bisphenol-A (BPA - 50μg/kg), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD - 0.036μg/kg) and their mixture (MIX), from gestational day 7 until birth. These dams were weighed weekly, but no significant differences in weight were seen. At five months post-delivery, dams underwent a glucose tolerance test. Only MIX exposed dams showed elevated blood glucose. In accordance cholesterol was altered, with MIX females having elevated total cholesterol, LDL, and free cholesterol. MIX dam's livers showed similar results with elevated total cholesterol and LDL. There was no overall difference in insulin concentration, however, control females trended towards an association such that high serum insulin indicated lower blood glucose. However, MIX females showed the opposite relationship, with elevated serum insulin significantly correlating with elevated blood glucose. These data suggest an inability of insulin, despite its elevation, to lower serum glucose concentrations in MIX dams. Metabolic biomarkers, adiponectin, MCP-1, PAI-1 total, and resistin were assessed with no differences observed in the MIX. These changes do not appear to be the result of acute hormonal changes, as serum steroid hormones (estradiol, corticosterone and testosterone) were not changed. These data provide biological plausibility for the epidemiological associations observed between EDC exposure during pregnancy and increased risk to develop Type 2 diabetes. Yet more data are needed on mechanisms of metabolic reprogramming (elevated blood glucose and cholesterol), nearly six months post MIX exposure. Such data are critical for public health prevention and potential intervention strategies focused on pregnancy as a critical window for woman's health.

2804 Polyphenols of Silverskin Coffee and Spent Coffee Produce Cytoprotection in Undifferentiated Neuroblastoma Cell Line

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Polyphenols have strong evidence of their beneficial impact on brain function during aging; in fact, caffeine has protective effects against Alzheimer's disease and Parkinson's disease and potential mechanisms to protect against blood-brain barrier leakage. Coffee is an excellent substrate for the growth of mycotoxins and polyphenols exert protective effect as demonstrated in *in vitro* assays. In this study, determination of polyphenols of coffee samples and effect on a undifferentiated neuroblastoma cell line, SH-SY5Y exposed to two mycotoxins α-zearalenol (α-ZOL) or beauvericin (BEA) were studied and three exposure strategies with two coffee by-product extracts (silverskin coffe and spent coffee) were assayed during 24 and 48h. Direct treatment consisted in exposing cells to pure coffee by-product extract or mycotoxins at 1:2 dilutions. Simultaneous treatment: extract at fixed dilution (1:4) of the coffee by-product extract and α-ZOL (50 μM) or BEA (2.5 μM) at dilutions 1:2. Finally, for pre-treatment procedure, cells were exposed as in direct treatment and after 24h cells were exposed to α-ZOL or BEA at dilutions 1:2. Direct treatment of α-ZOL and BEA increased cytotoxicity in SH-SY5Y cells in a time and dose-dependent manner, but only α-ZOL reached IC_{50} values of 25μM and 21μM at 48h and 72h, respectively. Pre-treatment with boiling water extract revealed an increase of cell viability for α-ZOL at 24 and 48h from 10 to 16% and from 30% to 25%, respectively with spent coffee; while with silverskin coffee a decrease was observed. Opposite to this happened for BEA where increase was observed for silverskin coffee at 24 and 48h, from 14% to 23% and from 25% to 44%, respectively; and decreased bellow 50% was observed for spent coffee. Comparison of different silverskin coffee extracts in pre-treatment and BEA revealed that boiling water cytoprotects SH-SY5Y cells either at 24h and 48h with viabilities ~100%. Considering the high viability of silverskin coffee extracts obtained these extracts can presents benefits as natural and sustainable food ingredient. *Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (AGL2016-77610-R) and Fondo di Ateneo per la Ricerca from Italy (FAR2018).*

2805 Mixture Designs to Investigate *In Vivo* Interactive Adverse Effects upon Co-exposure to Environmental Cyanotoxins

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Cyanobacteria are found ubiquitously in environmental water reservoirs and produce numerous toxins. Exposure to cyanotoxins has been linked to various neurodegenerative disorders, including sporadic amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases. Despite the known co-occurrence of various cyanotoxins, the majority of studies have focused on the investigation of a single cyanotoxin, a nonconical amino acid called β-methylamino-L-alanine, which poorly recapitulates an actual environmental exposure to cyanobacteria. A mixture design of experiment (DoE) was used to screen for interactive effects of various environmental cyanotoxins. Using a combination of viability and behavior-based toxicity assays (i.e., sensitivity startle assays), we evaluated possible interactions between BMAA and its isomers: 2,4 diaminobutyric acid (2,4DAB), aminoethyl glycine (AEG). In addition, we investigated microcystin LR (MCLR) which is known to co-occur with BMAA and its isomers in the environment. A dose response study for each cyanotoxin reveled that the cytotoxic mild-effect, which is defined as the LOAEL (<25% mortality), was 500 μM for BMAA and AEG, 250 uM for 2,4DAB, and 1uM for MCLR. Our results show the potential for interactions between BMAA and its isomers AEG and 2,4 DAB as well as BMAA and MCLR, which is highly prevalent in the environment. Exposure to cyanotoxic mixtures caused a significant increase in mortality rate in zebrafish larvae. Exposure to cyanotoxic mixtures also interfered with the innate threshold of the zebrafish larvae startle response ($p<0.05$, ANOVA), causing the zebrafish larvae to become more hyposensitive or hypersensitive to acoustic stimuli. Taken together, mixture design is a useful as a toxicology method to screen for interactions amongst cyanotoxins as cyanotoxic mixtures have the potential to interact in an *in vivo* system. We highlight the need to study cyanotoxic mixtures when investigating the link between cyanobacteria and neurodegenerative pathologies.

2806 Modeling Induction of Proximal and Distal Endpoints following PPARγ Activation by Ligand Mixtures

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Binding of chemicals to receptors and subsequent activation of transcription are the molecular initiating events (MIE) for a large number of biological processes. Based on this, we developed Generalized Concentration Addition (GCA), a pharmacologically-based mathematical model, to estimate the biological effect of mixtures. GCA successfully predicts experimental data for mixtures of AhR ligands and of PPARγ ligands. Commonly, dose response data used to test the efficacy of additivity models are generated in reporter assays. Here, we experimentally test the ability of GCA to model and predict endogenous biological events proximal (e.g. endogenous gene expression) and distal (e.g., differentiation) to PPARγ activation and to model the biological event distal from the MIE using the dose response data from the proximal MIE. We generated individual dose-response data for two full (rosiglitazone and S26948) and one partial (nTZDpa) agonists using the OP9 cell line (RRID:CVCL_4398). We assessed *Fabp4* mRNA expression after 24 hrs or lipid accumulation (a surrogate of adipocyte differentiation) after 5 days. The dose response curves for activation of PPARγ by rosiglitazone were largely overlapping, whether generated from reporter data, *Fabp4* expression or lipid accumulation. For all chemicals, proximal and distal endpoint dose responses were similar, with a slightly lower potency for induction of Fabp4 expression than lipid accumulation. We then generated mixture data with combinations of chemicals using a grid design. Combinations of full agonists induced maximal levels of Fabp4 expression and lipid accumulation. The partial agonist nTZDpa had agonistic effects at low effect levels and antagonistic effects to rosiglitazone at high levels, as predicted by pharmacological theory. We are comparing the empirical mixture data to that predicted by GCA from the individual dose response curves. Results from these studies will expand the applicability of generating and applying pharmacodynamics models of more complex biological endpoints in GCA.

2807 Impact of Plasma Protein Binding on the *In Situ* Hepatic Uptake and Clearance of Perampanel and Fluoxetine in Sprague Dawley Rats

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There is about 50% of drugs highly bound in plasma (fu_p <0.1) among new molecular entities approved by the FDA between 2003 and 2019, and it is yet unclear how to predict the hepatic clearance (CLh) of albumin-bound drugs with an affinity to other proteins. The main objective is to investigate the effect of albumin (ALB) and/or alpha-1-acid glycoprotein (AGP) on the hepatic uptake and CLh of the drugs that bind, extensively, to both proteins, and that are exclusively metabolized in the liver. From a drug data collection (n= 1907 drugs), two drug candidates are selected based on their physicochemical, metabolic and binding properties (Binding Ratio $_{AGP:\,ALB}$ close to unity): perampanel (PER) and fluoxetine (FLU). Using male Sprague Dawley rats (n= 3 rats/scenario/drug), isolated perfused rat liver (IPRL) experiments are performed to obtain intrinsic clearance (CL_{int}) and CLh values for the two drugs; at perfusate concentrations of 2.5, 7.5, 15, 20, 25 and 35 μM. There are four IPRL experimental scenarios: 1) physiological solution without plasma proteins (WO), 2) solution with bovine ALB (40 g/L), 3) solution with bovine AGP (1 g/L), and 4) solution with mixture of both proteins (MIX); at concentrations of 40 g/L and 1 g/L for ALB and AGP, respectively. The type of kinetics observed, and the metabolic rates are different for the two drugs. For the PER, four Michaelis-Menten kinetics are observed with Vmax= 225.23, 23.16, 112.13 and 17.32 (nmol/min/g liver) and Km= 24.85, 1.90, 37.0 and 0.90 (μM) for the WO, ALB, AGP and MIX, respectively. As for FLU, substrate inhibition (uncompetitive) kinetics are observed with Vmax= $2.84x10^5$, 67.7, $8.7x10^5$, 22.5 (nmol/min/g liver); Km= 205.6, 0.024, 518.4 and 0.0047 (μM) and Ki= 0.116, 0.424, 0.0004 and 1.4 (μM) in all four scenarios, respectively. The CL_{int} ratios (Vmax/Km) increased for PER but decreased for FLU in the presence of ALB and MIX compared to the WO and AGP scenarios. The presence of AGP decreased the CL_{int} ratios (Vmax/Km) for both PER and FLU compared to WO. Furthermore, in the rat liver, PER is poorly metabolized (hepatic extraction= 0.2-0.7), while FLU is highly metabolized in the different scenarios (hepatic extraction= 0.8-0.99). In conclusion, the ALB-mediated hepatic uptake is observed only for PER that is poorly metabolized, and for which the CLh would mainly depend on the CL_{int} that increased due to the binding to ALB. The FLU is a substrate inhibitor and highly metabolized, which makes it difficult to observe an uptake mediated by ALB for FLU since saturation of metabolic rate is not observable and CLh would mainly depend on the perfusate flow instead of CL_{int}.

2808 Estimating the Sensitivity of GARD™skin and GARD™air Assays for Evaluating Skin and Respiratory Sensitization Potential of Complex Mixtures; An Initial Case Study Using Spiked E-liquids

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Due to the dynamic nature of innovation with e-cigarettes, new assays are required to quickly determine the potential biological response of these products for stewardship activities. It is important to evaluate e-liquid ingredients and formulations for their potential skin and respiratory sensitising properties. We have previously shown that GARD™skin and GARD™air assays can be used to assess skin and respiratory sensitising potential of complex mixtures of experimental and commercial e-liquids. However, it remains to be explored if these assays can detect low concentrations of sensitisers within such complex mixtures. The objective of this study was to assess an experimental e-liquid spiked with different doses of sensitisers in GARD™skin and GARD™air assays. Both assays measure a genomic biomarker signature of a human myeloid leukemia-derived cell line exposed to test substances, and a prediction model is used to classify each sample according to its sensitising potential. An unflavoured experimental e-liquid (50:50 Propylene Glycol, Vegetable Glycerin, ± 1.6% Nicotine) was spiked with different doses of Eugenol (skin sensitiser) or Reactive Orange (respiratory sensitiser) in concentrations from 0-500μM. The experimental e-liquid by itself was determined to be non-sensitising; but became sensitising in GARD™skin and GARD™air, respectively, in a dose-response dependent manner when Eugenol or Reactive Orange were added to the formulations. Using a 4-parametric log-logistic dose-response curve an estimated threshold range for the induction of skin sensitisation in GARD™skin was estimated to between 1-50 μM (0.15-7.5ppm) for the Eugenol spike. Whereas, for GARD™air this estimated threshold range for the induction of respiratory sensitisation falls between 100-380 μM (88-330ppm) for the Reactive Orange spike. The detection of spiked sensitisers within e-liquids

allows us to estimate the minimal level of sensitiser detectable within a complex mixture and highlights the potential of this assay for future ingredient assessment strategies of e-liquids.

2809 Mutagenic Effects of Reference Cigarette 3R4F and E-cigarette Liquids with Different Nicotine Concentrations on L5178Y/TK$^{+/-}$-3.7.2C Mouse Lymphoma Cell

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Electronic cigarettes (e-cigarettes) are the most recently developed tobacco products, which are highly advertised across the media, mainly as healthy substitute to conventional cigarettes, aid in quitting smoking addiction or way of circumventing ban on smoking in public places. E-cigarette liquid (e-liquid) is mainly composed of glycerol, propylene glycol, nicotine and various flavors. It is found that the chemical composition of aerosol produced by e-liquid heating is quite different from that of traditional cigarette smoke, and the type and release amount of harmful components are significantly lower than that of traditional cigarettes. The mouse lymphoma assay (MLA) is used internationally for short-term mutagenicity tests, which has been considered as the most sensitive *in vitro* mammalian cell gene mutation assay and therefore is especially useful in evaluating substances with unknown or multiple genotoxic mechanisms.20 3R4F reference cigarettes were smoked according to the ISO condition (35 ml puff volume, 2 seconds duration, 60-second puff interval, with the ventilation holes unblocked). 3 commercial e-liquids with nicotine concentration respectively for 6, 12 and 18 mg/mL were smoked with the same e-cigarette device for aerosol production, according to the Health Canada Intense (HCI) condition (55 ml puff volume, 2 second duration, 60 seconds puff interval, with 100% blockage of filter ventilation holes). After smoking, the cigarette smoke condensate (CSC) and the e-cigarette aerosol extracts (ECEs) of cambridge filter pads were extracted with DMSO at 10 mg/mL and stored at -80 °C until analysis. All CSC and ECEs were studied in the presence of metabolic activation. The final S9 concentration in the treatment medium was 1.25%. The results showed that both the CSC and ECEs showed cytotoxic to the L5178Y cell. TFT resistance Mutation Frequency (TMF) of CSC was more than 3 times higher than that of the solvent control at higher concentrations (80-150 μg/mL), and induced dose-related mutagenic effects in L5178Y cells. There were no mutagenic effects of TK gene appeared in the ECEs in the dose range. Through derivation, the potency of nicotine in CSC (80 μg/mL) and ECE (320 μg/mL, Maximum exposure dose) are 1.536 and 2.365 mg respectively. There may be indicating that the mutagenic effect of the TK gene is mainly caused by the harmful components in the particle phase.

2810 *In Vitro* Assessment of Potential Chronic Toxicity of Smoke from Combustion of Flame-Retarded and Non-Flame-Retarded Furnishings

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To address concerns about potential chronic toxicity of smoke from flame retarded furnishings, smoke from combusted furnishings was examined in controlled room burns. Each room contained a cushion couch, chair and flat panel television of identical models/manufacturers purchased from either France, United Kingdom (UK), or US. The UK furniture met BS5852 fire standard and contained flame retardants. The French and US furniture met smolder only fire standards, e.g., TB117 2013, and were not flame retarded. As expected, vast differences were noted in ignition resistance and progression of fires. Smoke from the fires started as white and ended as dense black smoke. Smoke from both phases was sampled via XAD sorbent resin. Potential chronic toxicity of the smoke was assessed with both the ToxTracker reporter assay and Aryl Hydrocarbon Receptor (AHR) activation assay. ToxTracker consists of a panel of mammalian stem cell lines that contain different fluorescent reporters for induction of DNA damage, oxidative stress and protein damage. The differential induction of the GFP reporters as well as cytotoxicity of the tested substances was determined by flow cytometry. AHR transactivation assessed using a commercial reporter cell line. Two dark smoke and one white smoke sample from the French furnishings and one of the dark smoke samples from the US furnishings showed activation of the Bscl2-GFP genotoxicity reporter in ToxTracker which is associated with DNA replication stress and induction of pro-mutagenic DNA adducts and subsequent inhibition of DNA replication. None of the UK samples activated the Bsc12-GFP reported, however one UK black sample induced genes associated with oxidative stress. No other positive results were observed with ToxTracker. For AHR activation, only the two dark smoke and one white smoke samples from the French furnishings and one of the dark smoke samples from the US furnishings showed induction greater than 25% of the positive control, whereas all of the UK samples showed activation of less than 10%. Overall the data suggest that the combustion products from the more flame retarded furnishings showed a lower potential for chronic toxicity that those furnishings least flame retarded. *Supported by the American Chemistry Council's North American Flame Retardant Alliance.*

2811 Stability of Disinfection By-Products (DBPs) within the Complex Mixture Formed during Chlorination

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Disinfection of water with chlorine effectively reduces microbial contamination but also results in formation of complex mixtures of hundreds of DBPs. Speciation and concentrations of formed DBPs vary as a function of multiple factors, including: the concentrations of natural organic matter, bromide and iodide; time; temperature; pH; the disinfectant used, and the disinfection scenario. These combined factors create a non-steady state environment for DBPs that, coupled with lack of stability data, has hindered the ability to investigate potential health effects from exposure to environmentally realistic complex mixtures. In the present study, 75 regulated and priority DBPs along with total organic halide (TOX), an integrated measure of the total mass of organic halide formed during chlorine disinfection, were monitored by sampling over the course of a comprehensive rat multigenerational reproductive toxicity study (EPA's Four Lab Study). In an effort to enhance stability, the chlorinated water concentrates were provided to the rats by a watering system that held the concentrates dark, cold, and head-space free. Concentrates were analyzed over time for the 44 individual DBPs plus TOX for which at least two sampling events confirmed detection above their respective analytical minimum reporting limits (MRLs). Stability was evaluated by testing the hypothesis that the slope across time of logarithmically transformed DBP concentrations was zero. The criterion for stability was lack of statistical significance when compared to a Bonferroni adjusted significance level of 0.05. The four trihalomethanes (chloroform, bromodichloromethane, chlorodibromomethane, bromoform) regulated by EPA were stable over 14 days. Of the five haloacetic acids regulated by EPA, three were stable (di- and tri-chloroacetic acid, dibromoacetic acid) while two (chloroacetic and bromoacetic acid) were below their MRLs. Overall 39 of the 44 DBPs analyzed for stability met the criterion for stability, as did TOX. In sum, these results indicate that the majority of the evaluated DBPs were stable over the course of the study.

2812 Identification of Co-occurrence Patterns of Persistent Organic Pollutants with Data Mining Methods

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Environmental contaminants occur in mixtures, but traditional toxicology and risk assessment focus on single chemicals. Understanding risk associated with exposure to mixtures requires knowledge of those chemical combinations that occur together most frequently. Our objective was to use Frequent Itemset Mining (FIM), a data mining technique, to find the most commonly occurring combinations of items in a dataset. We identified a dataset where 50 persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides, were measured in fish sampled from 540 sites on US rivers. With the POPs from this study, over one quadrillion possible mixtures exist. The goal was to identify candidate mixtures that appear in fish in 25% or more of the river sites. Chemical occurrence data were filtered using three separate methods into presence/absence matrices. The three separate limits indicate different confidence levels for analyte concentrations being greater than zero. These limits, in order of increasing tightness, are: any amount detected, method detection limit (MDL, 99% confident analyte is present), and quantitation limit (QL, 3 x MDL). Using these thresholds, three sets of frequent combinations were identified: 14,290,875 combinations by any amount detected, 611,876 combinations by MDL, and 745 combinations by QL. For all three limits, the 30 most frequently occurring combinations comprised of either two,

three, or four chemicals and appeared in 46-88% of all river sites, and the most frequently occurring mixture by each method was 4,4'-DDE + PBDE-47 (88% any, 87% MDL, 68% QL). Even though the number of combinations decreased as the threshold became more stringent, 21 of the top 30 most frequently occurring mixtures remained the same. Larger mixtures with at least 8 chemicals present were identified: 12,795,381 by any detection, 401,534 by MDL, and 3 by QL. In summary, while infrequently applied to environmental contaminant studies, FIM has strong potential to identify mixtures of concern based on combination frequency. *(This abstract does not reflect US EPA policy).*

2813 Evaluating Toxicity of a Representative Mixture from a Legacy Creosote Site Affected by Wildfire Smoke

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Polycyclic aromatic hydrocarbons (PAHs) are a highly abundant group of organic chemicals with numerous man-made and natural sources. Inhalation of PAHs from various sources can have negative impacts on individuals' lung health due to certain PAHs having toxic, mutagenic, or carcinogenic properties. PAHs are formed from the incomplete combustion of sources such as coal, oil, gas, and wood. Increases in temperature and changes in precipitation, as a result of climate change, are increasing wildfire prevalence and severity. Wildfire smoke contains complex mixtures of pollutants such as particulate matter, PAHs, and volatile organic compounds (VOCs). An estimated 339,000 deaths each year (260,000 - 600,000 deaths annually) are attributed to wildfire smoke, however the overall impact on respiratory and cardiovascular health are largely unknown. PAHs can also be found in the air as a result of chemical movement between environmental compartments at contaminated sites such as legacy creosote sites. Creosote oil is a wood preservative derived from coal tar, which is composed of a complex mixture of hundreds of compounds including VOCs and PAHs. However, the primary components of creosote oil that contribute to adverse health effects have not yet been identified. In the summer of 2017 stationary air passive samplers were deployed at a legacy creosote site during the Eagle Creek Fire in Northern Oregon. Sample extracts were analyzed for 63 PAHs using GC/MS-MS. A representative mixture of the top 6 most abundant PAHs (Mix6) from 5 different stationary air samplers was formed at environmentally relevant ratios. The Mix6 was then evaluated for pulmonary toxicity using primary normal human bronchial epithelium cultured at the air liquid interface. Toxicity of the Mix6 was evaluated by measuring tight-junction integrity, cytotoxicity and induction of xenobiotic metabolizing and oxidative stress enzymes. Exposure to the Mix6 resulted in significant (<0.05) induction of transcriptional biomarkers of oxidative stress and xenobiotic metabolism, NQO1 ($p=0.0353$) and CYP1A1 ($p=0.0051,0.0271$), indicating potential mechanisms of toxicity. The toxicological profile of Mix6 will be further evaluated by comparing it to its individual components. Future studies will include conducting benchmark dose modeling in order to evaluate levels at which the Mix6 elicits pulmonary toxicity. Results from this study will help inform relative health impacts from exposure to mixtures of PAHs from inhalation of wildfire smoke and/or living in proximity to a legacy creosote site, and elucidate mechanisms of toxicity of PAH mixtures.

2814 Application of Retention Index in Analysis of 241 Tobacco Flavor Components by Gas Chromatography-Tandem Mass Spectrometry

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Tobacco flavor Components are very important to the aroma and taste of tobacco products and greatly influence the quality, characteristics and acceptability of the finished product. The high selectivity and sensitivity of gas chromatography tandem mass spectrometry (GC-MS/MS) with dynamic multiple reaction monitoring (dMRM) mode can satisfy the qualitative analysis of trace level flavor components in complex tobacco matrices. However, the target retention time may drift out of the acquisition time window after the instrument maintenance and method adjustment, and retention time correction is time consuming, especially in the multi-analyte analytical method. In this study, retention index (RI) was used to quickly obtain the retention times in a GC-MS/MS method for the simultaneous analysis of 241 flavor components. The retention indices of 241 target components were determined by detecting tobacco samples spiked with a mixture of n-alkanes and target standards. The difference values of retention indices between experimental determination and NIST17 database information were less than 10 for 211 components, and the retention indices of 30 components were not reported previously. Then, the retention times of the target compounds under different chromatographic conditions were predicted based on the retention indices of targets and the retention times of n-alkanes mixture, and the results showed that the predicted retention times were very close to the experimental measured ones. The time deviations of 232 components were within 0.05 min, and the time deviations of 9 components were within 0.1 min, which can fully meet the requirements of dMRM acquisition time window. It is proved that the retention index has good stability in the complex tobacco matrix, and can be used in the retention time prediction in multi-analyte GC-MS/MS analytical method, and it's accurate and time saving.

2815 Formulation Development and Analysis Methods for Polycyclic Aromatic Compound Mixtures in Support of Toxicology Studies

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Polycyclic aromatic compounds (PACs) are ubiquitous environmental contaminants which typically occur in mixtures. PACs are of particular concern due to their long life in the environment and potential routes of exposure, such as ingestion, occupational exposures (eg. tar, asphalt etc), and indoor (cookstove emissions) and outdoor pollution (diesel exhaust). 13 PACs and 3 mixing ratios were selected based on individual toxicity data and predictions about mixture effects; an equimolar combination of the individual PACs, the ratio of each PAC at the dose eliciting 50% effect (ED50), and the ratio of each PAC at the dose eliciting 10% effect (ED10). The PACs included, benzo[a] pyrene, phenanthrene, pyrene, acenaphthenequinone (ANQ), benzo[k]fluoranthene, benzo[c]fluorene, dibenzo[a,l]pyrene (DBP), dibenzothiophene, dibenz[ah]anthracene (DBA), chrysene, benz[j]aceanthrylene (BJA), benzo[b] fluoranthene, and indeno[123-cd]pyrene. The goal of the work reported here was to develop methods to prepare and analyze PACs in corn oil formulations. Due to the complexity of the formulations (number of chemicals with similar properties, and the wide concentration range of the test articles), two stocks, one containing 7 and one containing 6 of the chemicals were prepared in corn oil. Serial dilutions of these stocks were prepared and the actual formulations were prepared by combining the dilutions of the stocks. The same analytical method could not be utilized for all compounds. Therefore, the two separate stocks were analyzed for the individual PACs and the combined mixture (formulation) by High Performance Liquid Chromatography with fluorescence detector (all except ANQ) and Gas Chromatography-Flame Ionization Detector (ANQ) for marker compounds. The formulation analysis methods were validated over the concentration range 8.0-38,400 ng/mL (compound dependent). Standard curves were linear ($r^2 > 0.98$) and the method was accurate (relative error (RE) $\leq \pm 15$ %) and precise (relative standard deviation (RSD) $\leq$ 10%) for all concentrations except at the lowest concentration for DBA (RSD $\leq$ 30%) and BJA (RE $\leq \pm 11$%). The formulations were homogeneous for all PACs (RSD $\leq \pm 5$%, except DBP (RSD $\leq \pm$ 15%)). The formulations stored at 2-8°C were stable up to for 44 days except ANQ. In conclusion, we have successfully developed formulation and analysis methods in support of the toxicity assessment of PAC mixtures.

2816 Simultaneous Determination of 69 Aldehydes and Ketones in Tobacco by Gas Chromatography Tandem Mass Spectrometry

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Aldehydes and ketones are very important tobacco flavor Components, which greatly influence the quality and characteristics of the tobacco product. Sensitive and selective multi-analyte analytical methods are needed to satisfy the demand for analyzing trace level flavor constituents in tobacco. A reliable and rapid gas chromatography tandem mass spectrometry (GC-MS/MS) method for the simultaneous analysis of 69 aldehydes and ketones in tobacco was developed and validated using a modified QuEChERS extraction procedure. The mass spectrometry parameters, including parent ions, product ions and collision energy, were optimized. The impact of extraction solvents, soaking conditions, soaking duration, vortex duration and purification methods on extract efficiency were also investigated. Dynamic multiple reaction monitoring mode (dMRM) was applied for the quantification and confirmation of those compounds. The results showed that the calibration curves of all the targets presented good linearity. Average recoveries of all of the compounds in tobacco ranged from 70.1 to 120.1% with relative standard deviations of 0.3-19.7% at three fortification levels. The limits of quantification (LOQs) and the limits of detection (LODs) for 69 aldehydes and ketones were in the range of 2.9-136.9 µg/kg and 0.9-40.9 µg/kg at the signal-to-noise ratio (S/N) of 10 and 3, respectively. The method greatly improved the detection performance

and the range of target compounds, and can meet the requirements for aldehyde and ketone flavor constituent analysis in tobacco with the advantage of accuracy, sensitivity and convenience. The experimental method was successfully applied to the analysis of 15 cigarette samples with different style characteristics, and 55 aldehydes and ketones were detected. A total of 5 compounds with p-value less than 0.01 were screened out as differential components. It further proves the practicality of the developed analytical method.

2817 Assessing Additivity of Chemical Effects at the Gene Expression Level in Complex Mixtures of Environmentally Relevant Chemical Concentrations

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Exposure to complex mixtures at environmentally relevant concentrations can affect both human health and ecological endpoints. The cumulative effects of these complex mixtures are often estimated by assuming simple additivity; however, the additivity of chemical effects at the gene expression level remains poorly understood. We test for evidence of additivity in environmentally relevant concentrations of complex mixture at the gene expression level by comparing differentially expressed genes from exposures to 7 mixtures of up to 20 chemicals, as well as their single chemical constituents, using zebrafish (*Danio rerio*) embryos as a model system. The gene expression profiles of the individual chemical constituents were summed to create theoretical gene expression profiles for each mixture under the assumptions of additivity. We compared differentially expressed genes in theoretical mixtures to empirical measurements to test the hypothesis that exposure to complex mixtures at environmentally relevant concentrations will cause gene expression to change in an additive manner. A Spearman's rank correlation (rho) test was used to compare differentially expressed gene profiles and revealed very weak correlations between empirical and theoretical mixtures (median rho=0.04, maximum rho=0.15). Further analysis of empirical and theoretical mixture comparisons revealed no tendency towards synergism or antagonism in gene expression, and up to 60% of differentially expressed genes were expressed in opposite directions. Thus, we did not find evidence to support simple additivity of effects from exposure to complex mixtures at the gene expression level in zebrafish embryos. This work highlights that low-dose components of environmentally relevant mixtures may not act additively. *The views and opinions expressed are those of the authors and do not necessarily reflect the official policy or position of the US Army. This work was supported by the US Army's 6.1 Basic Research Program, Environmental Quality and Installations.*

2818 Associations of Endogenous Reproductive Hormones and Phthalate Exposure with Subjective Sleep Quality in Midlife Women

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Impaired sleep quality during the menopausal transition reduces quality of life, increases depression, and increases risk of metabolic diseases. Endogenous hormones in midlife women are known to influence subjective sleep quality. Endocrine disrupting chemicals, such as phthalates, may also contribute to the increased prevalence of sleep disturbances in this population. Phthalates are known to impact endogenous hormones, including reproductive hormones, that are associated with sleep. However, the link between phthalate exposure and sleep quality remains unexplored. Thus, we investigated associations between phthalate exposure, serum reproductive hormones, and subjective sleep in the Midlife Women's Health Study (MWHS), a longitudinal cohort from Baltimore, Maryland and surrounding counties. We hypothesized that increased exposure to environmentally relevant phthalate mixtures is associated with impaired sleep in midlife women. Additionally, we hypothesized that this association is mediated by reproductive hormones. Using ordinal logistic regression, we assessed the associations of both phthalate mixtures, measured from urine, and serum reproductive hormone levels with subjective sleep quality. Sleep was measured using survey questions, where participants indicated frequency of sleep disturbances, insomnia, and restless sleep. Statistical models were adjusted for BMI, hot flashes at night, menopause status, CES-D score, and present quality of life, and were stratified by smoking status. We found that progesterone is negatively associated and estradiol:progesterone (E:P) is positively associated with all measures of sleep quality in nonsmokers. Further, we found that the sum of phthalates associated with personal care products (sumPCP) and sum of all phthalates (sumALL) are negatively associated with all sleep measures in former smokers. There was no significant mediation effect of progesterone or E:P on the association between sumPCP or sumALL and sleep quality. These data indicate that reduced progesterone levels and a larger E:P are associated with impaired sleep, and increased sumPCP is directly associated with reduced frequency of sleep impairments. This is one of the first studies to identify associations between phthalates and sleep and adds insight into the role of endocrine signaling in mediating adverse symptoms accompanying menopause. *Supported by NIH R01ES026956.*

2819 *In Vitro* and PBPK Models for a Mixture of Dioxin Like Compounds

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Mathematical modeling is an important tool in the development of *in vitro* to *in vivo* extrapolation methods. In support of extrapolation from cells to organisms, we wanted to evaluate a smaller step of extrapolation from cell model to physiologically based pharmacokinetic (PBPK) model. We developed PBPK models and *in vitro* system models that share the same detailed pharmacodynamics. The PBPK models are for TCDD, PeCDF and PCB126 as well as a mixture of all three. The *in vitro* model is for hepatocytes and has the same liver kinetics equations as the PBPK models. While the PBPK models yield accurate predictions of kinetic data from the NTP Dioxin Mixture 2-year studies, the *in vitro* model using PBPK parameters fails to match literature data. We show where there are discrepancies going from the PBPK model to the *in vitro* model.

2820 Joint Toxicity Assessment of Perfluoroalkyl Substances (PFAS) Mixtures

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Humans are exposed to multiple perfluoroalkyl substances (PFAS), through a combination of intake of food, water, dust particles, and hand-to-mouth transfer. Communities across the country are becoming increasingly aware and concerned about the presence of these chemicals in their bodies, but the mechanisms of toxicity of PFAS have not been well understood. The 2018 ATSDR mixtures framework recommends three broad approaches for toxicity assessment that are also now used by other federal and state agencies. Of these, the most often used is the hazard index (HI) approach that is based on potency weighted additively of the toxicity of individual components. Recent experimental toxicity studies of mixtures containing perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) as well as an assessment of 17 PFAS found in the general population revealed no major concern for joint toxicity based on the HI approach. ATSDR conducted risk assessment of binary mixtures of PFAS for multiple toxicity endpoints applying this approach. Using estimated lifetime exposures reported in the literature, we determined that a 2-component hypothetical mixture of PFOS and PFOA will yield hazard indices (HIs) of 30, 2.01, and 0.215 for high-exposure scenarios; 8.4, 0.13, and 0.102 for intermediate-exposure scenarios; and 3.63, 0.034, and 0.047 for low-exposure scenarios for developmental, reproductive, and thyroid toxicity, respectively. In these calculations, we utilized the intermediate duration minimal risk levels and target organ toxicity doses. The HI values are used as a screening and prioritization tool, the concern for exposures increase as the HI values increase. For HI values >1, a follow-up including uncertainty factor analysis is conducted. Until further insights are gained in their mechanistic toxicology, based on our current knowledge, the HI approach appears most suitable to address present day PFAS public health concerns. However, this approach must be used cautiously because of the uncertainties related to the large toxicity data gaps that exist for an overwhelming majority of PFAS. *The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

2821 Thyroid Receptor Antagonism of Mixtures Isolated from Personal Silicone Wristbands

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Given the inherent difficulties with collecting and assessing human biospecimens, there is a critical need for external, non-invasive samples that can recapitulate human exposures. Silicone wristbands have been shown to support measurements of personal exposure to complex mixtures of semi-volatile

contaminants in the ambient air, encompassing both the indoor and outdoor environments. We previously demonstrated that extracts of household dust promoted thyroid receptor beta (TRβ) antagonism and differentiation of pre-adipocytes *in vitro* and found that these bioactivities were significantly associated with thyroid hormones and body mass index of adult residents. Herein, we conducted a pilot study to evaluate TRβ antagonism of mixtures isolated from silicone wristbands worn by 36 adults recently diagnosed with thyroid cancer and 36 age and sex-matched controls from central NC. Adults wore wristbands for one week, then they were wrapped in foil and frozen until analysis. One-gram sections of the wristband were solvent extracted and analyzed via mass spectrometry to quantify brominated and organophosphate flame retardants. Separate extracts were reconstituted in tissue culture media for use in bioassays and tested for their ability to antagonize TRβ, using a reporter gene assay in human kidney cells. Approximately 70% of extracts tested at 1% (concentration of extract in contact with the cells) exhibited significant TRβ antagonism, with no activity in wristband blanks. These effects occurred independent of toxicity, as measured via lactate dehydrogenase release and constitutive promoter reporter assays. Higher extract test concentrations (5 and 10%) did exhibit significant toxicity. Extracts exhibited a mean of 30% TRβ antagonism (% suppression of half maximal positive control) and a range of 0-100% antagonism at 1% concentration, and a mean of 10% with a range of 0-63% at 0.1%. Thyroid receptor antagonism was positively correlated with wristband concentrations of 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB) and tris (1-chloro-2-propyl) phosphate (TCPP). These results suggest that these personal passive samplers may be useful in evaluating bioactivities of mixtures that people come into contact with on a daily basis; however, more research is clearly needed to understand the drivers of this activity, and to determine if mixtures agonize or antagonize other nuclear receptors.

2822 E-cigarettes: Partnering with Scientists to Enhance Community Engagement

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According to the CDC, an estimated 5 million US middle and high school students reported using e-cigarettes in 2019, an increase of 1.4 million youth over 2018. This trend has raised concern in public health circles about youth addiction to nicotine from e-cigarette use and unknown respiratory effects of e-liquid constituents such as flavorings. It has also highlighted a need for youth and community education about the potential health effects of e-cigarettes. In response, researchers investigating the effects of e-cigarette solvents and flavorings on respiratory host defense at the University of North Carolina (UNC) at Chapel Hill partnered with the UNC Center for Environmental Health and Susceptibility's Community Engagement Core (CEC) to engage public health professionals, K-12 audiences, and families in the toxicology of e-cigarettes. For the CEC, partnering with scientists deepens community engagement by providing educational content that is real, relevant and robust; for scientists, partnering with community engagement experts can enhance research endeavors and science communication skills. In this presentation, we will share strategies for effectively involving scientists in community engagement activities and K-12 education by providing examples from the CEC's work to engage broad audiences in learning about the potential health effects of vaping. We will describe (1) hands-on activities developed for use at public science events and (2) a data interpretation activity developed for high school biology students in which students examine evidence that e-liquids and aerosols impair the respiratory immune system. E-cigarettes provide biology students with a relevant context in which to explore and refine what they know about cell structure and function, the immune system, and cellular respiration. We will highlight the process for developing a lesson that incorporates published scientific data and introduces students to the field of toxicology. Though these projects are specific to e-cigarette research, they serve as examples for how scientists in all areas of toxicology can partner with informal science educators to reach broad audiences by developing standards-aligned K-12 and public science education materials.

2823 Integrating New Technologies into High School Toxicology Education

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Early introduction to the fields of toxicology and environmental health is critical for the development of the next generation of scientists. Towards this effort, the Rutgers Toxicology, Health and Environmental Disease (THED) summer program was established six years ago to provide high school students an opportunity to explore scientific careers and an introduction to principles and approaches fundamental to toxicology. Recently, we integrated interactive technologies into the program. Students were assessed on how effective the technology was in improving engagement, learning, and the overall educational experience. Poll Everywhere, a classroom response system was incorporated into presentations on forensic toxicology, histology, and pathology. Students anonymously answered multiple choice and open-ended questions, participated in word clouds, and responded to clickable image questions in real time using cell phones or laptops. This provided a way to engage students and assess their understanding of the materials being taught; it also provided feedback to instructors on teaching effectiveness. During the summer, THED students also observed a simulation of a patient undergoing cardiac arrest using a high-fidelity simulation mannequin in the Rutgers School of Pharmacy Acute Care Simulation Suite. They also toured the School's Parenteral Sterile Products and Community Pharmacy Simulation Laboratories where they learned about the preparation of intravenous and solid dosage forms of medications and counseling of pharmacotherapy plans, respectively. Using the Likert scoring system (1 low, 7 high), students rated the EMSOP simulation tour experience a mean of 6.4 (0.8 SD, N=53). The pharmacy simulation in the Acute Care Simulation Suite was the highest rated activity for the program overall. Students also commented that they enjoyed the experience and found it relevant to their future career decisions. Overall, the integration of technology into the THED program aided in student engagement and provided real time feedback assessing achievement of learning outcomes. *Supported by NIEHS T32ES007148, P30ES005022, and U54AR055073.*

2824 Lettuce Not Be Salty*:* An Update of a Common Secondary Education Experiment Measuring Seed Germination under Salt-Stressed Conditions

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Many high school and college classrooms conduct experiments to assess the environmental implications of using salt as a deicer on roadways. Most commonly, this experiment has been completed with sodium chloride as the salt, and seed germination is measured from buttercrunch lettuce seeds. However, as we are becoming a more environmentally conscious society, the use of salt alternatives is becoming more common. Thus, the aim of our work was to update the traditional lettuce seed toxicity experiment to account for two new sodium chloride alternatives and assess their effect on seed germination. For our alternatives, we choose calcium chloride and sodium chloride enhanced with calcium magnesium acetate and a beet juice additive (i.e. beet salt). Calcium chloride is advertised as being more pet-friendly and can be purchased both online and in local and national pet stores. In addition, beet salt is advertised as being less harmful to the environment because it contains the natural ingredient of beet juice and is enhanced with calcium magnesium acetate, which does not contain harmful chlorides. Syracuse, NY - one of snowiest big cities in the United States - launched a pilot program in 2010 to use beet salt on one of its major highways to supplement rock salt, and in 2018 Syracuse started using beet salt on the city's sidewalks to clear the walkways of ice. This suggests that its use may become more common as cities move towards different, more-environmentally friendly salt alternatives. We did a dose- and time-response experiment, utilizing 5 different concentrations of the salts while growing the lettuce seeds for up to 5 days. We found that the lettuce seeds grew the best in the beet salt solutions and the worst in the sodium chloride solutions, with comparable seed growth between the water control and calcium chloride solutions. With discernible differences in growth between the salt types, our results suggest that this experiment can be successfully updated to examine differences between the effects of different salts on plant life in order to assess and discuss the environmental implications of salts and their derivatives on roadways.

2825 Toxicology for Chemists: A Curriculum Project Connecting Toxicology to Chemistry Education

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A key sustainability challenge today remains that molecular designers (chemists) are not trained how to address hazards at the very beginning, design stage of a product life-cycle. Chemists lack training in toxicology and the understanding of what makes a molecule hazardous to human health and the environment. This knowledge gap results in chemical products that have unintended consequences. Beyond Benign, a non-profit dedicated to Green Chemistry education, has launched a new curriculum project - Toxicology for Chemists - that is aiming to address this knowledge gap. The project involves the development of curriculum and resources that support current and future scientists (chemists) to better understand molecular hazard how to include the information within their design criteria as molecular designers.

A host of curriculum materials and on-line resources are currently being created that will remain open access for instructors, students and scientists to design chemical products that are safer and healthier for humans and the environment. The Toxicology for Chemists program is guided by a key group of advisors, comprised of professional toxicologists and chemists. Throughout the project, the curriculum will be bridging the two subjects - toxicology and chemistry - and connecting key learning objectives from each discipline. By providing much needed toxicology resources for chemists, this will enable scientists to better design and prepare safer alternatives that have reduced human and environmental hazards.

2826 Expanding the Reach of Undergraduate Education Programs at Regional Meetings: Model Programs at Four Regional SOT Chapters

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Regional meetings are excellent opportunities for undergraduate students to learn about toxicology and to interact with a variety of professional toxicologists. However, the scientific programs at these meetings are often at depths beyond an undergraduate audience, and absent targeted recruiting, undergraduates typically attend only if recommended by an SOT member adviser. The Faculty United for Toxicology Undergraduate Recruitment and Education (FUTURE) committee supports regional chapters with grants and materials to provide undergraduate programming. Several features of undergraduate-focused programming at the SOT Annual Meeting have been successfully adopted at the regional meetings. "Lunch With an Expert" provides an opportunity for undergraduates to interact with toxicologists from alternative career paths to academia, such as industry or government. Undergraduate poster sessions provide an opportunity to present data that is more preliminary, preparing them to later present at national conferences through practice and critical feedback. An undergraduate breakout session has also proven popular among attendees. An "Introduction to Toxicology" slide set provides an overview of career paths and active learning exercises such as the "Opioids" activity have been used at two regional meetings. Both are available at the SOT website in the Learning Resources area for undergraduate educators. A keynote speaker can provide a career-focused talk that describes a pathway to becoming a professional toxicologist. Participants are subsequently encouraged to apply for the national meeting's Undergraduate Diversity Program, or, if they have an abstract, the Pfizer SOT Undergraduate Travel Award. A new unified online program assessment survey was used to provide undergraduate feedback for different aspects of each program for the four regional chapters awarded grants in 2019-2020. Likert scale and free-form responses indicate high satisfaction with all programs, as do comments such as "enjoying meeting people", "enjoyed keynote speakers discussing toxicology and education presentations", and "enjoyed undergraduate breakout session."

2827 Revision of Introductory Toxicology Course and Applications beyond the Classroom in Sierra Leone

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Environmental toxicology is a persistent issue in Sierra Leone in large part due to inadequate waste management, rapid population growth, and the release of high levels of hazardous materials into the environment. As a result, Sierra Leoneans are highly susceptible to environmentally-related deaths. While access to education has steadily improved over the last two decades, many adults in Sierra Leone lack resources and tools to combat persisting environmental challenges. Specifically, many members of the government and industry are in positions of influence yet operate with little to no understanding of the basic toxicological problems that plague their communities. In 2018, our group developed the first ever toxicology course taught in Sierra Leone. This course was designed using evidence-based teaching practices, including the incorporation of active learning activities, minimal lecturing, and group work to promote a growth mindset and learner-centeredness. These concepts were tailored towards college students in Sierra Leone such that we employed case studies and local examples that are relevant to this unique environment. The course was taught in the Spring of 2019 and drew nearly 20 students of both graduate and undergraduate standing. Students successfully completed the course by submitting a research paper detailing the science behind a toxicant of interest. After receiving feedback from the students and using the recent publication of the learning framework for undergraduate toxicology courses as our guide, we modified the curriculum to align with the research-backed learning objectives as published (Gray et. al, 2019). The curriculum was also modified to target a broader audience, specifically members of the community such as government officials and industry workers that would benefit from a toxicology education. While the materials were developed for a semester long course, we modified them to be taught in a weeklong certificate program. We retained many of the core concepts of toxicology while also maintaining the learner-centeredness of this the original course. We believe that both the workshop and course model can be applied in other developing countries allowing for more widespread dissemination of the core toxicology concepts.

2828 Team Science in a Summer Undergraduate Fellowship: Field Sampling for Metal Contamination in the Raritan River

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Summer internships provide undergraduate students with intensive one-on-one training experiences in the laboratory. At Rutgers University, we sought to extend this training to promote scientific collaboration and networking through team-based field sampling. During the 2019 Summer Undergraduate Research Fellowship (SURF) Program, twelve undergraduate students collected surface water from the Raritan River aboard the R/V Rutgers following completion of interactive classroom sessions on heavy metal contamination. The river has a history of extensive pollution from industrial facilities and includes multiple Superfund sites along its shore. In collaboration with the Marine and Coastal Sciences program, surface water was collected in duplicate at 6 sites between New Brunswick, NJ and the Raritan Bay. Students kept field notes on sample characteristics as well as weather and water conditions. ICP-MS analysis revealed a large range of metals concentrations in the sampled surface water. Lithium (mean: 33.0 ppb), barium (mean: 20.8 ppb), and arsenic (mean: 16.2 ppb) were the most abundant metals analyzed, while lead (mean: 0.06), cadmium (mean: 0.02 ppb), and cobalt (mean: 0.48 ppb) were the least abundant. Notable changes in metal concentrations were observed in relation to whether samples were collected near and away from industrial and landfill sites, and may provide clues on the primary sources of contamination. Concentrations of lithium, vanadium, chromium, cobalt, nickel, copper, arsenic, cadmium, cesium, and uranium increased up to 14-fold in samples collected between the Edison landfill and Sayreville brownfield, with an overall trend of increasing concentrations toward the Raritan Bay. By comparison, barium decreased toward the Raritan Bay, while lead was highest upstream from the major contamination sites, but remained constant throughout the remaining the stretch of river. Participants rated the surface water sampling activity highly (mean rating = 4.6, SD 0.5) on a Likert scale of 1-5. The students' overall impression of fieldwork was that it was fun to perform research outside, and that the experience provided tangible relevance of toxicology to their lives. *Supported by the SOT Intern Program, R25ES020721, P30ES005022, and T32ES007148.*

2829 Digital Storytelling in Biology: Increased Engagement and Inclusiveness through a Novel Assessment Method

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Effective active learning pedagogies enable students to identify and solve problems that inspire and intrigue them. While life sciences courses offer content featuring exciting challenges and achievements, common assessment mechanisms--exams and quizzes--frequently fail to inspire or to facilitate deep learning. Rather, they discourage many students, leading them to drop the course or even leave the discipline. This phenomenon disproportionately affects students from underrepresented backgrounds. Here, we describe a digital storytelling initiative, used in place of a comprehensive final exam, in an introductory biology course. Student teams generated a short video narrative ("digital story") to propose a gene editing strategy for curing a heritable disease and to evaluate the feasibility of its implementation in patients. In-class workshops and evening help sessions were employed to teach students to use bioinformatics databases relating to the course modules (genetics, molecular cell biology, evolution). Students then exercised their new skills to investigate the genetics and molecular mechanisms of a heritable disease and develop a gene editing strategy. Each digital story integrated multiple course concepts and inspired students of diverse backgrounds and test-taking abilities to view material through the lens of their own interests and motivations. We hypothesized that digital storytelling would increase student investment

and confidence as well as achievement of learning goals and retention. To assess the project's effectiveness, we used student surveys and enrollment data. Survey responses were demonstrably enthusiastic, both for the project content and for the opportunity it provided to demonstrate creativity and deep learning. Remarkably few students left the course or received failing grades. We suggest that digital story projects represent an effective learning strategy that could be readily applied to topic reviews, mock grant proposals, and case studies in introductory toxicology courses, and can increase inclusiveness and retention by promoting student engagement. *Support: Kenyon College Center for Innovative Pedagogy and HHMI Inclusive Excellence Initiative.*

2830 Benefits of Near-Peer Mentoring: The Near-Peer Perspective

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Undergraduate research experiences that offer individual participation in scientific research programs can play a key role for preparing our next generation of scientists and health care professionals. A key component of a successful research experience is mentoring which is often facilitated by faculty members, but may also involve near-peer mentors (i.e., graduate students). While mentoring relationships provide substantial benefits to the mentee, benefits that may be obtained by a near-peer mentor are not well-documented. The Summer Undergraduate Research in Environmental Health Sciences (SURES) program at the University of Kentucky is a 10-week program that offers research experiences to rising junior and senior undergraduates. Mentoring is provided by both faculty and near-peer mentors (NPMs). The NPM are recruited and selected by the program director, participate in a mentoring training session and are then matched to their respective mentee following a "speed mentoring" event. The NPMs are asked to meet weekly with their mentees and participate in several weekly SURES events. To evaluate the NPM experience, we conducted pre- and post- program surveys. Our survey results indicate that the top three reasons that NPM participated in the SURES program were overall self-improvement, to improve their teaching and mentoring skills and to develop undergraduate students. In working with their mentees during the 10-week period, they reported focusing their efforts on ensuring that the mentees would feel comfortable contacting them and talking with them as well as feeling emotionally supported. The top benefits of the mentoring experience reported by the NPM were gaining experience and skills in mentoring and coaching, and leadership. NPMs also reported they improved their communication and interpersonal skills, learned about current undergraduate students, and further determined their own career interests. The results from this study provide insights into the benefits provided to NPM who engage in a near-peer mentoring relationship that would contribute to their professional career development.

2831 A Native American Tribe and Its Continuing Toxic Legacy

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In the early 1960s through the late 1970s, millions of gallons of paint sludge and other toxic materials were dumped by a large car manufacturing plant into abandoned mines, forested areas, and nearby homes in Ringwood, NJ. As a result, sections of Ringwood became riddled with toxic metals in their soil, water and air, placing a large environmental burden on the very people who have lived on this land for generations - the Ramapough Lunaape Turtle Clan Nation. Upon community request, samples of soil, water, plants and fish tissue were collected from community-identified sites by citizen scientists and NYU graduate assistants to evaluate soil metal concentrations from this "remediated" 500 acre Superfund site. Soil samples were collected by digging 3-5 inches deep with a plastic shovel and placed into individual zip lock bags stored at room temperature until analyses. Each dried soil sample was analyzed in duplicate using two independent X-ray florescence (XRF) instruments. Twenty heavy metals, including lead (Pb), arsenic (As) and cadmium (Cd) were analyzed and their levels compared to NJ Direct Contact Soil Standards for dermal, ingestion, and inhalation exposure. Both As and Pb were found in several different soil samples collected around the local Ringwood church at levels exceeding the NJ soil standard of 19 mg/kg and 400mg/kg, respectively. Besides being a carcinogen, ingestion of As has been associated with increased cardiovascular and respiratory diseases. Ingestion of Pb, particularly by children causes decreased neurocognition, hyperactivity, lower IQ scores, and immune suppression. The greatest concern about soil contamination arises primarily from health risks from direct contact with the contaminated soil and secondary contamination of water supplies within and underlying the soil. Given the Native American children who continue to play in this area and the Tribes' tradition of growing their own food, this finding is of serious concern for these already-vulnerable Indigenous people. *Supported by NYU NIEHS P30 Center ES-000260-55.*

2832 Measuring Residential Socioeconomic Factors Associated with Pollutant Releases Using the US EPA Toxic Release Inventory

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Many environmental justice studies claim that the population groups most exposed to disproportionate levels of pollution from polluting facilities are minorities and low-income individuals. This study sought to test that claim against four counties in upstate New York: Albany, Erie, Monroe, and Onondaga Counties. These counties are located within 300 miles of one another, and all contain major Upstate NY cities, including Albany, Buffalo, Rochester, and Syracuse, respectively. Onondaga County is infamous for its presence of polluting facilities, and their associated chemical releases; this is seen particularly with Onondaga Lake, at one point the most polluted lake in the United States. The four selected counties for this analysis are comparable to one another with respect to size, population, geography, and demographics, allowing a larger geographic area to be examined for pollutant releases, while still looking at similar population information. Nine population identifiers assessing residential socioeconomic status (R-SES) were obtained from the 2000 US Census at the block group level. The demographics were grouped together, based upon their similarities, using a hierarchal clustering method. The method produced seven unique residential clusters that were then mapped across the four counties. The location of each Toxic Release Inventory (TRI) facility within the four counties was then geocoded against the clusters to determine which population characteristics, if any, were more influential in deciding whether a facility was located near a group of individuals. It was seen that minorities and low-income individuals were not disproportionately exposed to these polluting facilities. Instead, we found consistently that the largest predictor of whether a polluting facility was found in a geographic location was for laborers working in non-managerial positions. Our findings could be due to companies considering available and skilled labor when siting their facilities. Moreover, the R-SES cluster with one of the highest percentages of laborers in non-managerial positions,78.59% of residents, released over 10 million pounds of chemicals in 2000 alone. The next highest amount of chemical releases by any R-SES cluster was just under 3 million pounds. This suggests those in non-managerial positions are not only disproportionally located near these facilities, they are also exposed to higher levels of pollutants.

2833 A Pilot Project to Apply a Collaborative Framework to Brownfields Redevelopment in Northern Kentucky

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A brownfield is a site formerly used for industrial or commercial purposes where future use could be affected by contamination. The goal of this pilot project was to apply and further develop a collaborative framework to enhance community engagement in chemical risk assessment and decision-making for contaminated site redevelopment. The project began with an intensive search of the scientific literature to identify best practices in community-engaged research and risk communication. We also reviewed publicly available documents on over 300 potential redevelopment sites in Northern Kentucky. Following consultation with environmental experts and government agencies, we narrowed the list of sites to 10 for in-depth consideration. The site furthest along with redevelopment plans was the Marianne Theater, a historic building in Bellevue, KY. We are now working with city leaders and residents to develop messaging regarding the building and its value to the community. We will rely on information gained about three other historic theater projects from other area of Kentucky as well as data gathered on successful and unsuccessful brownfield redevelopment projects. The ultimate goal is to identify critical success factors and obstacles that hinder effective redevelopment. A key lesson learned in the early stage of the project was the importance to communicate more than environmental information to gain wider acceptance of a project within a community and to ensure stronger financial support to complete the remediation and renovations.

2834 Needs and Opportunities to Develop Cost-Effective and Safe Dressings to Treat Chronic Wounds in Lima, Peru

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Chronic wounds are a burden for health systems worldwide and deplete health resources despite the existence of evidence indicating that self-care prevention reduces hospitalizations and emergency visits. Healing rates range between 21 and 35% and the recurrence rate is large, leading to high bed occupability, excessive treatment costs and negative effects on quality of life. Therefore, there is a need to innovate in the treatment of wounds of difficult resolution from clinical and health services management perspective. This work analyses interviews with experts in the Metropolitan area of the city of Lima, Peru to identify problems arising when treating chronic wounds. On average, the interviewees stated that they serve ten patients in bed per month and seven patients per outpatient clinic. Thanks to this information, we estimate that in Lima alone there would be at least 16,200 patients with chronic wounds. Likewise, the interviewees stated that they use about ten hydro-colloid or hydro-active dressings per month, which allows estimating that there is an annual demand of 5.8 million dressings. When analyzing the primary interests of stakeholders, it was concluded that their main concerns are the healing time and the bad smell generated by the wounds of the patient. In summary, we conclude that this is a new market in the country, which means that there are no leaders and the market is divided by the different existing commercial products; however, it is the hydrogels that are beginning to position themselves in this market. Thus, a strategic alliance should be made with proactive doctors working in the public sector and private sector revolutionaries to magnify the reception of innovative dressings in the city of Lima, Peru.

2835 Dietary Exposure and Health Risk Estimation of Polycyclic Aromatic Hydrocarbons (PAHs) Levels in Nile Tilapia from a Tropical Creek

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Increasing levels of persistent organic pollutants in aquatic ecosystems is a major concern globally due to the potential adverse effects to ecological receptors and humans via the food chain. The study assessed the risk associated with dietary exposure to polycyclic aromatic hydrocarbons (PAHs) in muscle tissues of Nile tilapia, *Oreochromis niloticus* from Agboyi creek in Southwest-Nigeria. PAHs were determined using Gas Chromatography-Mass Spectrometry (GC-MS) following EPA methods. Of the 16 priority PAHs detected, the mean concentration of Acenaphthene (60.51 ± 69.85 µg/kg) accounting for 19 % of total PAHs was the most predominant while Benzo (a) pyrene (0.08 ± 0.17 µg/kg) accounting for 0.03 % of total PAHs was the lowest. Estimated daily intake (EDI) of PAHs through consumption of fish were higher than reference dose (RfD) indicating high risk. The results of Hazard Index (HI) > 1 also emphasized potential negative health effects in consumers. Furthermore, the toxic equivalent concentration (TEC) values for Benzo (b) fluoranthene (35.79 µg/g) and Dibenz (a, h) anthracene (56.25 µg/g) which exceeded USEPA screening value of 0.67 ng/g and the estimated excess cancer risk (ECR) for 7 most toxic PAHs detected in fish tissues suggests that life time exposure in adult consumers would result in cancer risk.

2836 Canaries in the Coal Mine, Canines on the Couch: A Model for Investigating Contaminant Exposures to Support Human Health Research

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Only 5-10% of human cancers can be explained by genetics alone, suggesting the environment plays a strong role in disease etiology. Quantifying the impacts of exposures remains challenging due to latency issues that can take years to manifest after exposure. Dogs may provide valuable insights as a sentinel species for exposure-related human disease because they experience similar environment exposures, have a 6-8 fold shorter lifespan, share many clinical and biological behaviors, and have closely related genomes. We evaluated individual exposures among pet dogs and their paired human companions using silicone dog-tags and wristbands as personal passive samplers (n=30 pairs). Silicone samplers were analyzed for a suite of chemicals across multiple compound classes, including organophosphate esters (OPEs), polybrominated diphenyl ethers, polychlorinated biphenyls, phthalates, and pesticides. As a validation pilot study, we collected urine samples from each study participant and dog, and measured levels of OPE metabolites. 32 of the 41 compounds measured, with a detection frequency >50%, were significantly correlated between dog and human wristbands (r_s = 0.38-0.90; p <0.05), indicating the dog could be a valuable One Health model and potential sentinel species for examining how exposure to consumer product chemicals impact health. The concentrations of several OPEs parent compounds measured on the dog tags were significantly correlated with their respective metabolites in urine (r_s = 0.50-0.71; p <0.01). These data support the value of using the domestic dog as a sentinel species to investigate the potential long-term health impacts on humans from shared exposures.

2837 The Modified Molisch Reaction for Quantitative Determination of Bisphenols

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Bisphenol A [BPA or 2,2-bis(4-hydroxyphenyl)propane] is a high volume chemical mainly used in manufacturing of polycarbonate plastics. Exposure to BPA has been associated with endocrine disruption, birth defects and metabolic syndrome. Among other sources of exposure, contact with thermal paper used at cash registers can be a significant source of BPA exposure and absorption through the skin. We have recently shown that the Molisch test, extensively used for the detection of sugars, with minor modifications can be used to detect BPA in thermal cash receipts. However, since the Molisch test and its modification are just the ring tests with low detection limits, we have examined if the modified Molisch test can be a basis for a quantitative determination BPA and other bisphenols, *viz*., bisphenol-F (BPF), bisphenol-S (BPS), and bisphenol-Z (BPZ). To establish optimal conditions for the quantification, experiments were performed with different sugars (source of furfural derivative) and bisphenols, all being incubated in a strong acid medium at 45±2 ^{0}C. Typically, 0.2 ml of 1% sugar solution (glucose or galactose in water) was mixed with 2 mL of cold 75% H_2SO_4. A 20 µL-aliquot of bisphenol (BPA, BPF, BPS or BPG; ; 0 to 1 mM) in 70% ethanol was added while contents were mixed thoroughly. The final mixtures were heated for periods up to 60 min in a water bath at 45±2 ^{0}C. The samples at various intervals were cooled to the room temperature and read in a Geneses 10S UV-Vis spectrophotometer between 400 and 700 nm. The results were surprising in that, of the various bisphenols studied, only BPA gave the characteristic purple product with a broad absorption maximum centered around 564 nm. In the case of BPA, the product formation reached a maximum by about 35 min and remained constant for periods beyond 60 min. The $\xi_{564\ nm}$ for the BPA reaction product was found to be 35,000 $M^{-1}cm^{-1}$ (detection limit: 0.5 µM). While the exact reasons for bisphenols other than BPA not forming the purple product are not clear, we believe the oxidation of the furfural condensation product with these bisphenols was too slow or negligible. This proposition was confirmed by the apparent lack of purple ring formation in the modified Molisch test performed with other bisphenols used. Apart from providing a sensitive method for determination BPA, the results of BPS not forming a purple product are truly significant given the fact most manufactures of thermal paper are now shifting their attention to BPS or other bisphenols and that BPS, as reported in some recent studies, was present in the samples of human breast milk. Thus, the present study can be of significance to know presence of BPA as an adulterant in other bisphenol formulations.

2838 Suspect Screening for Urinary Metabolites of Imidacloprid after a Single Oral Dose in a Human Volunteer by HR-MS

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Imidacloprid (IMI) is one of the world's most widely used insecticides. Despite its global use for more than 20 years, only little is known about its metabolism in humans. However, exposure and risk assessment of IMI using human biomonitoring requires sensitive and specific biomarkers of exposure and knowledge of their respective urinary excretion fractions. Therefore, this study intended to investigate the human metabolism of IMI and to identify urinary

metabolites potentially applicable as biomarkers of exposure. After a single oral dose of 5 mg IMI to a male volunteer, urine samples were collected for 48 h (IRB Reg No. 18-6680-BR). The obtained samples were subjected to enzymatic deglucuronidation and analyzed using LC-Q-Orbitrap-MS with subsequent data mining using a commercial software. In a preliminary analysis, screening hits were identified based on the accurate masses of their (protonated and deprotonated) molecular ions, including ^{13}C and $^{35}Cl/^{37}Cl$ isotopolog signals with corresponding signal intensities. Hits with plausible excretion profiles (peak area vs. time) were included in a confirmatory analysis using data-dependent acquisition of product ion spectra. Screening hits were confirmed as tentative urinary metabolites of IMI if the major mass signals in the product ion spectra could be explained by plausible fragment ions. 6-Chloronicotinic acid, a compound which has been previously suggested to be a metabolite of IMI and other neonicotinoids in humans and, consequently, has been already used for exposure assessment, could not be detected in this first screening. Non-metabolized IMI was found in all urine samples. Moreover, two phase-I metabolites previously described in rats, 5-hydroxy IMI and IMI-olefin, were identified. These metabolites together with their $^{13}C_2$,^{15}N-isotope-labeled analogs have been synthesized for further verification by HPLC-MS/MS. Our results show for the first time specific biomarkers that are actually most suitable for human biomonitoring of IMI.

2839 Aggregated Exposure to Di(2-ethylhexyl) phthalate from Foods and Cosmetics through a Physiologically Based Pharmacokinetic (PBPK) Model and Comparison with Measured Urinary Metabolite Concentrations: Results from the EuroMix Biomonitoring Study

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A human biomonitoring (BM) study was performed to study exposure of chemicals present in foods and personal care products (PCPs). For two 24-hour study periods separated by 2-3 weeks, adult volunteers (44 males and 100 females) in Norway kept detailed diaries on food consumption (type/brand, weight, time and packaging material) and the usage of PCPs (type/brand of product, time and number of applications). In parallel, 24 hours urine samples were collected. Monte-Carlo simulation was carried out by Oracle Crystal Ball ©. This software calculates exposure based on the propagation variable of variability and uncertainty given by each parameter in a probability function, until a certain number of iterations. Individual external aggregated exposure to di(2-ethylhexyl) phthalate (DEHP) aggregating dietary with non-dietary exposure from PCPs was calculated. External exposure estimates were used for internal dosimetry simulation using physiologically based pharmacokinetic (PBK/PBPK) models. Simulated 24 hours urinary concentrations were compared with BM of the urinary metabolites of DEHP, mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl 5-carboxypentyl phthalate (MECCP). The results show that diet is the major contributor to DEHP exposure for both males and females, with an external exposure approximately 10 times higher than for the exposure from personal care products (PCPs). The main contributors to the mean dietary DEHP exposure are milk and dairy products, with a percentage contribution of 69.3 % and 62.8 % of the total exposure for males and females respectively. Additional food groups contributing to DEHP exposure were grains and grain-based products, fruits and vegetables, meat and fish. For both males and females, deodorants contribute most to the exposure of DEHP from PCPs, with a 79 % and a 53 % contribution, respectively. Verification of the exposure data using forward-dosimetry PBK/PBPK model give good convergence with 24 hours urinary concentrations of simulated and measured BM data. The measured concentration of the MECCP metabolite seems to correlate well with the simulated high exposure, while the measured concentrations of MEHP, MEHHP and MEOHP were more dispersed and partly overlapped with both simulated low, medium and high metabolite exposure.

2840 Aggregate and Product-Specific Exposures to Chemicals from the Use of Consumer Products

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Many chemicals are present in more than one consumer product. As a result, an individual will receive multiple doses of a chemical from use of different products and their aggregate (total) dose from all products will exceed the dose received from any single product. The Chemical Human Exposure Model (CHEM) evaluates residential exposures to chemicals having consumer product sources using a population-based, longitudinal model that employs a Monte Carlo simulation method. CHEM simulates the combinations of products used by a household, the composition of the products, and the chemical exposures to an individual that result from the use of the products. We used CHEM to investigate the relationship between product-specific and aggregate doses in the general adult population in the United States. Six compounds known to be used in many types of products were evaluated: propylene glycol; isopropyl alcohol; methyl paraben; linalool; limonene; and di-sodium EDTA. Daily systemic doses were estimated for 1,000 adults for each day of a simulated year. Each adult's dose from their, or another household member's, use of a consumer product was determined and summed to give the aggregate dose for the adult. The model outputs were used to estimate measures of chronic exposures (annual average daily dose) and acute exposures (largest daily dose). The impacts of uncertainty in product composition were also investigated. The number of products used by an individual in a given year that contained a chemical ranged from 0 to 14. The 95% lower confidence limits in the six chemical's doses were less than 10^{-6} mg/kg and the 95% upper confidence limits ranged from to 0.1 to 3 mg/kg. CHEM predicted that chronic average and acute daily doses were similar in size. This occurred because many of the products containing the compounds were used on a daily basis. For insight into the relative importance of chemical exposures from multiple products, the Maximum Aggregate Ratio (MAR), defined as the ratio of an individual's aggregate doses to their largest product-specific doses, was computed. Values of MAR for both chronic and acute exposures of the simulated adults had median values of less than 1.2 for all chemicals and did not exceed 2.7 for any individual and chemical. MAR values declined with increasing aggregate doses for both acute and chronic exposures. These findings suggest that for the six chemicals, adults with the largest exposures had aggregate exposures that were similar in size to their largest product-specific doses. This pattern is consistent with the patterns observed in cumulative exposures to multiple chemicals for humans and environmental receptors.

2841 Characterization of Aerosolized Particles Generated from Machining of Nanoclay-Enabled Composites

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Nanoclay-enabled polymer nanocomposites (NPCs) are poised to dramatically impact composite technologies across numerous applications. Release of airborne particulate containing organomodified nanoclays (ONC) at key steps along the NPC life cycle represents an emerging occupational inhalation exposure hazard. This study hypothesized that different types of surface organic coatings, percent ONC loading, and machining process would impact the physicochemical characteristics of generated airborne particulate. Two different ONCs, Cloisite 93A and Cloisite 25A, were embedded in polypropylene (PP) at three different percent loading (0%, 1%, and 4%). Each NPC was characterized for ONC dispersion and strength properties. Next, NPCs were sanded in a controlled exposure chamber with different sanding belt types to generate airborne particulate, which was measured in real-time. Inhalable and respirable fraction samplers were deployed to collect particles for electron microscopy analyses to quantify particle characteristics. ONC type and percent loading influenced NPC strength and toughness, which significantly correlated with particle release. Both 1% ONC and 4% Clois93A NPCs exhibited significantly greater strength, toughness, total particle number, and respirable mass concentration than 4% Clois25A and virgin PP NPCs. A majority of mass from machined NPCs was within the inhalable fraction. A noticeable peak in the respirable fraction (<30 nm) was detected and varied in magnitude, with 1% NPCs producing more ultrafine particulate. ONC type and load influenced the released NPC dust particle size, mass, and elemental composition, which was a complex mixture of NPC and sand particulate. ONC was primarily found protruding from or embedded within NPC composite particles. In summary, abrasion of NPCs produces particulate in the inhalable fraction with 1) protruding ONC platelets and 2) noticeably different concentrations of

sanding particles depending on ONC type, loading, and NPC strength characteristics. These findings implicate the potential for airborne particle exposure during manipulation of NPC materials.

2842 Exposure Assessment of Traffic-Related Air Pollution in El Paso, Texas, Using Personal and Ambient Monitoring

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El Paso is home to one of the largest border crossings in the US and has also been in nonattainment of the National Ambient Air Quality Standards (NAAQS) for particulate matter in the past. Significant sources of emissions have been identified in the US-Mexico border region, where large volumes of cross-border freight and passenger movement occur. These emissions are due to a large number of heavy-duty vehicles/trucks which are often moving slowly or idling while waiting in long lines at the border. The objective of this exploratory study was to characterize human exposure to traffic-related air pollution in El Paso, utilizing both personal and ambient air monitoring. We measured real-time personal exposure to fine particulate matter ($PM_{2.5}$) and black carbon among high school teachers in El Paso using lightweight backpacks containing air monitoring devices over 24 hours. The ambient monitors were placed within the school premises and measured daily $PM_{2.5}$ and black carbon concentrations. Preliminary results from the real-time personal air monitors show the average daily mass concentration of $PM_{2.5}$ was 88.35 μg/m^3, (SD=270.89) and of black carbon was 1.09 μg/m^3, (SD=0.86) but in the absence of one outlier with very high levels of $PM_{2.5}$, the average for $PM_{2.5}$ became 2.68 μg/m^3 (SD=3.34). From the ambient monitors, the average daily mass concentration of $PM_{2.5}$ was 8.98 μg/m^3 (SD=5.45), and for black carbon was 1.12 μg/m^3 (SD=1.67). These low personal exposure levels may be as a result of the teachers spending most of their workday indoors, within the school and infiltration shielding offered by the building. However, further analysis of the filters from the personal air monitors is ongoing to reveal the actual concentrations and the implications for public health.

2843 Longer Commutes Are Associated with Increased Human Exposure to Tris(1,3-Dichloro-2-Propyl) Phosphate

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Organophosphate esters (OPEs) are a class of semi-volatile organic compounds (SVOCs) used as flame retardants, plasticizers, and anti-foaming agents. Due to stringent flammability standards in vehicles and the ability of OPEs to migrate out of end-use products, elevated concentrations of OPEs have been found in car dust samples around the world. As many residents of Southern California spend a significant amount of time in their vehicles, there is a potential for increased human exposure associated with longer commute times. With approximately 70% of the University of California, Riverside's undergraduate population commuting, the objective of this study was to use silicone wristbands to monitor personal exposure to OPEs by commute time for a subset of these students. Participants were asked to wear wristbands for five continuous days and complete daily surveys about the amount of time spent in multiple modes of transportation, and these data were then used to calculate a participant-specific total commute score. Phthalates contributed to the vast majority (97%) of total SVOC mass present on the study participant wristbands, followed by OPEs, brominated flame retardants, polychlorinated biphenyls/pesticides, and fluorotelomer alcohols. Components of the commercial flame retardant mixtures Firemaster 550 (triphenyl phosphate, or TPHP, and isopropylated triaryl phosphate isomers) and Firemaster 600 (TPHP and tert-butylated triaryl phosphate isomers) were strongly correlated with other OPEs found within the same mixture. Moreover, tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) was significantly correlated with certain Firemaster 500 components and tris(2-chloroisopropyl) phosphate (TCIPP). Finally, out of all SVOCs measured, TDCIPP was the most strongly and significantly correlated SVOC ($p<0.0001$) with total commute score, indicating that longer commutes are associated with increased human exposure to TDCIPP. Overall, our findings raise concerns about the potential for chronic TDCIPP exposure within vehicles and other forms of transportation, particularly within densely populated and traffic congested areas such as Southern California.

2844 Exposure Assessment and Micronuclei Induction in Populations Occupationally and Environmentally Exposed to Electronic Waste in South-West Nigeria

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Informal electronic waste (e-waste) reprocessing in Nigeria is reportedly substantial in Africa, putting the growing exposed population at high risk of metal toxicity. The existence and or extent of chromosomal aberration or genotoxicity indicators in the growing e-waste exposed populations in Nigeria have not been documented. In this cross-sectional study, 632 consenting participants were recruited from South-West Nigeria; consisting of 381 e-waste workers (EWW), 120 environmental e-waste exposed participants (EEP) and 131 age-matched unexposed participants (UP), serving as controls. A validated structured questionnaire was used to assess exposure pattern, the Inductively Coupled Plasma-Mass Spectrometry was used to determine toxic metal levels in whole blood while frequency of micronucleated polychromatic erythrocytes (MNPCE)/1000PCE in peripheral blood film was determined by modified Micronucleus Assay. Occupational and environmental exposures were high for EWW; environmental WEEE exposure which was moderate for the EEP, was minimal for UP. A duration of exposure of ≥ 5 years and exposure frequency ≥ 6 hours/day; 6 days/week (9360 hours in any 5 year duration) was observed with both EWW and EEP. Routes of exposure observed in EWW entailed eyes, oral route, nasal cavity, and skin. In addition, the proportion of EWW that used personal protective equipment (PPE) while working was barely 10.24% while non - PPE users constituted the majority (89.76%) of the studied population. Blood levels of the toxic metals (lead, arsenic and cadmium) were significantly raised. The frequency of MnPCE/1000PCE in EWW (22.70 $\pm$ 0.15) was significantly higher than EEP (4.17 $\pm$ 0.28), which in turn was significantly higher than the lowest frequency (0.99 $\pm$ 0.76) observed in UP. The observed exposure pattern and the comparatively higher MN induction in the e-waste populations may suggest risk of significant cytogenetic damage and aberrant chromosomal changes associated with occupational e-waste reprocessing in Nigeria.

2845 Urinary Blood Lead Clearance and Its Relationship to Glomerularfiltration Rate Based on a Large Population Survey

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Decreasing glomerular filtration rate (GFR) in association with increasing blood lead levels (BLL) or bone lead (Pb) concentrations have been reported in epidemiological studies of Pb workers and general populations. A complicating factor in studies that rely on BLL or bone Pb as exposure metrics for studying GFR is that alterations in GFR may directly affect Pb body burden by changing metal clearance. In 2009, the National Health and Nutrition Examination Survey (NHANES) began reporting data which enabled calculation of paired blood Pb clearance (CbPb) and serum creatinine clearance (CsCr), a metric of GFR, for individual subjects. Estimates of CbPb and CsCr were determined for approximately 7,600 subjects with data provided by NHANES for survey years 2009–2016. Eligible subjects included those with Pb concentration in blood and urine, creatinine concentration in serum and urine, urine flow volume(s) and time(s) (to estimate urine flow rate), gender and age. Subjects with GFRs < 10 or >1000 L/day were omitted under the assumption that the GFRs outside of this range indicated disease or potential errors in the data. Median CbPb in adults was 0.04 L/day (5th-95th percentile range: 0.01–0.12). Stepwise linear regression models for CbPb were estimated to identify significant explanatory variables. Variables explored in stepwise regression included BLL, CsCr, and several variables that were significant in linear regression models of CsCr (age, gender, body weight, and height). Regression models explained approximately 68% of variance in CbPb in adults, with >98% of explained variance attributed to CsCr. Significant predictors ($p<0.05$) for adolescents included CsCr, gender, age, and height (R^2 = 0.67), and CsCr accounted for approximately 95% of the explained variance in CbPb. Values for variance inflation factors were <1.6 for all independent variables in all models, indicating multicollinearity was not an issue with the regression models. Our study is apparently the first that quantified individual subject CbPb and GFR in a large sample. The slope for the relationship between CsCr and CbPb predicts a nearly 1:1 relationship between GFR and CbPb where 10% a change in CsCr is associated with a 10% change in CbPb. These results provide an improved understanding of the possible effects of reverse causation in the interpretation of studies of associations between blood Pb and GFR.

2846 The Chemical Landscape of High-Throughput New Approach Methodologies for Exposure

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The rapid characterization of risk to humans and ecosystems from commercial chemicals requires information on both hazard and exposure. The US EPA's ToxCast program and the interagency Tox21 initiative have screened thousands of chemicals in various high-throughput (HT) assay systems for *in vitro* bioactivity. EPA's ExpoCast program is developing complementary HT methods for characterizing the human and ecological exposures necessary to interpret HT hazard data in a real-world risk context. These new approach methodologies (NAMs) for exposure include computational and analytical tools for characterizing multiple components of the complex paths chemicals take from their source to human and ecological receptors. These pathway components include chemical use, release, transport, toxicokinetics, and, ultimately, external and internal exposure. Exposure NAMs being developed in ExpoCast include machine learning models that draw inferences from existing data, statistical frameworks that integrate predictions from multiple models, and non-targeted analytical screening methods that generate new HT monitoring information. Here, we evaluate the landscape of exposure NAMs in the context of various chemical lists of scientific and regulatory interest, including the ToxCast and Tox21 libraries, Toxic Substances Control Act (TSCA) inventories, and larger libraries of structures recently curated within EPA's CompTox Chemicals Dashboard. We show that exposure NAMs drastically improve the coverage of the chemical landscape compared to traditional approaches. While existing HT approaches provide comprehensive coverage of many key chemical inventories for human receptors, additional work is needed to develop analogous predictions for ecological receptors. A critical bottleneck is the prediction of pathway-specific chemical releases, especially ambient releases to the environment. *In vitro* toxicokinetics information is needed for additional substances to refine exposure-dose modeling and expand the domain of *in silico* models. The utility of exposure NAMs has been demonstrated in limited case studies on household dust and consumer products, rapidly increasing the number of chemicals identified in these media. The application of exposure NAMs to additional media will be critical for the improving the scope of evaluation datasets for HT exposure predictions.

2847 Assessing the Effectiveness of a Residential Metals Abatement Program (RMAP) Using Blood Lead Levels in Children

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The Butte RMAP program begin in 1994 and by the end of 2018 sampling of soil and dust was completed for 3,332 out of 3,646 residential parcels in the Butte Priority Soils Superfund site in Montana, with 1,152 properties and attics abated. To assess the effectiveness of the RMAP in reducing lead exposures, a 2014 study (Phase 1) evaluated blood lead levels (BLLs) in children ages 1-5 during 2003-2010. National Health and Nutrition Examination Survey (NHANES) blood lead data for the applicable years were weighted to match the Butte population for key variables known to affect BLLs and was used as a reference population. During the study period, Butte BLLs declined more rapidly than those of the NHANES reference population, and by 2009-2010 Butte geometric mean BLLs were no longer significantly different from NHANES. An update to this study (Phase 2) includes 2,330 BLL records from 2012 through 2017. The blood lead limit of detection (LOD) is 3.3 µg/dL for Phase 2, with BLLs measured using a point-of-care device, compared to the LOD of 1 µg/dL for Phase 1 data. Almost 80% of BLLs in Phase 2 are below the current LOD so remediation effectiveness is focused on evaluating the percent of children with BLLs greater than the reference level of 5 µg/dL. Among children 12 to 60 months of age, 172 of 2,330 (or 7.4%) tested between 2012 and 2017 had BLLs greater than 5 µg/dL. Older neighborhoods with more historical mining influence show a higher percentage of samples greater than 5 µg/dL in both study periods, and the percentage of BLLs above 5 µg/dL were about 20-30% higher on average in the warmer half of each year. House age was the most influential variable in the Phase 1 study, but information on house age is no longer collected as part of the more recent NHANES so could not be compared in the Phase 2 study. While this factor and the much higher detection limit for the Phase 2 Butte blood lead data prevent direct comparison of rates of elevated BLLs between NHANES and Butte, comparison of the rates of change in the elevated BLLs between the two datasets can still be conducted. Between 2003 and 2016 the percentage of the BLLs in Butte greater than 5 µg/dL descended at a faster rate than did the NHANES BLLs. Both of the Phase 1 and Phase 2 data show a leveling off in the later years (2013 to 2016) with higher rates persisting in Butte. Due to the very old housing stock and the higher poverty rate among the Butte population, substantial differences in lead risk factors between the Butte and the NHANES populations are likely to persist even when remediation is complete.

2848 Investigating Sensitization Activity of Azobenzene Disperse Dyes via the Direct Peptide Reactivity Assay (DPRA)

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Azobenzene disperse dyes used to color synthetic fabrics have been characterized in literature as immune sensitizers. However, little is known about human health implications of these dyes in indoor environments. Scarcity of data is due partly to the lack of purified reference standards. Our research group and others have found that azobenzene disperse dyes are widely present in house dust, raising questions about their potential to behave as immune sensitizers at environmentally relevant levels indoors. Here, we report on sensitization activity of azobenzene disperse dyes known to be present in children's clothing and indoor environments at environmentally relevant concentrations. To address the lack of standards, individual dyes were extracted from 13 commercial dye mixtures via Soxhlet extraction, followed by purification using CombiFlash Rf+ Chromatography (Teledyne ISCO, Hexane:EtOAc). Purity and identity were evaluated using high-performance liquid chromatography (HPLC) with diode array detection (DAD), high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance spectroscopy (NMR). We confirmed the chemical structures of 15 dye compounds for use as reference standards. Because covalent binding to nucleophilic proteins is a strong indicator for chemical sensitization potential, we used a spectrophotometric *in vitro* Direct Peptide Reactivity Assay (DPRA) to measure covalent protein binding via peptide depletion induced by azobenzene disperse dyes. Twenty dyes were tested: the 15 in-house purified compounds as well as commercially-available dyes Disperse Orange 25, Disperse Orange 37, Disperse Orange 61, Disperse Blue 373, and Disperse Violet 93. Results were confirmed via an HPLC-based reference DPRA to evaluate performance of the spectrophotometric DPRA for this application. Preliminary results demonstrate that dyes such as DO61 bind to nucleophilic protein residues and induce up to 41% (± 1%) peptide depletion at 0.2mM in a dose-dependent manner. This is notable when compared to characterized sensitizers such as 1-chloro-2,4-dinitrobenzene, which induces 100% peptide depletion at 10mM but induces only 16% depletion at 1mM (Wareing et al. 2017). Results suggest that exposures to azobenzene disperse dyes may present sensitization risks, warranting further detailed characterization.

2849 Dietary Lead and Phosphate Interactions Affect Oral Bioavailability of Soil Lead in the Mouse

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Low level exposure to lead (Pb) in early life has profound and long-lasting health effects. Widespread use of this toxic metal has resulted in extensive and persistent contamination of soil and dust. Soil and indoor dust are major sources exposure of children to Pb. A critical determinant of the extent of internal exposure to Pb through ingestion of soil or dust is its bioavailability, the fraction of ingested Pb that crosses the gastrointestinal barrier and is available for systemic distribution. Effects of dietary phosphate (P) level on the oral bioavailability of Pb present in soil were examined in a mouse model. Adult female C57BL/6 mice had free access to AIN-93G purified rodent diet amended with Pb as a soluble salt, Pb acetate, or in a soil matrix (NIST SRM 2710a) with a certified Pb concentration of 5520 mg/kg. In these studies, the basal diet contained P at a nutritionally sufficient level (0.3% w/w), with approximately equal amounts of P contributed by potassium phosphate in the diet's mineral mix and by phosphoproteins in casein, the diet's protein source. The modified diets contained P at a lower (0.15%) level by omission of potassium phosphate from the mineral mix, or a higher (1.2%) level by increasing the amount of potassium phosphate in the mineral mix. Pb speciation analysis was performed for samples of diet and feces using X-ray absorption spectroscopy. Relative bioavailability was estimated as the ratio of linear regression slopes for relationships between cumulative Pb dose (mg) and tissue Pb level (mg/kg of bone or kidney, mg/l of blood). The linear regression slopes were estimated by simultaneously fitting test (NIST SRM 2710a) and reference (Pb acetate) group tissue data to linear regression models sharing common intercepts. For either dietary Pb source (Pb acetate or NIST SRM 2710a), low dietary P level markedly increased accumulation of Pb in bone, blood, and kidney. Tissue Pb levels in mice fed a high P in diet were not different from

mice fed the basal P diet. Dietary P and Pb interacted to affect body weight change and feed efficiency in mice. The relative contribution of different Pb species in diet and feces was also affected by dietary P level. Differences in Pb species between diet and feces indicated that transformation of Pb species can occur during gastrointestinal tract transit. These interactions between Pb and P that alter Pb speciation may be important determinants of the bioavailability of Pb ingested in soil.

2850 Comparisons of $PM_{2.5}$ and $BC_{2.5}$ Concentrations in the Subways of Cities in the Northeastern United States

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Subways move millions a people a day across the world, bringing people to and from work and leisure. They are essential to the working economy of many cities. More are expected to be built, globally. We know that particulate matter (PM) levels on platforms are many times higher than ambient, but we know little of the origin of this pollution. No other study has investigated intercity differences in subway air quality in the Eastern United States. Here we report the $PM_{2.5}$ and $BC_{2.5}$ concentrations in the subways in Philadelphia, Boston, Washington, and in the MTA and PATH run stations in the New York City metropolitan area. We monitored real-time concentrations with a pDR 1500 and a microaethalometer on a total of 83 platforms in 74 stations across 12 lines during the morning (6:00 - 10:30 AM) and evening (3:00 - 7:00 PM) rush hours. We also collected aerosol particles on quartz and Teflon filters at the stations with the two highest measured $PM_{2.5}$ concentrations measured in each city; we calculated the organic carbon (OC) and elemental carbon (EC) and the $PM_{2.5}$ concentrations with each form of filter, respectively. Comparisons between city, time of day, on- and off- train, and train line were made. Mean real-time $PM_{2.5}$ concentrations in underground stations were 391.91, 250.45, 144.88, 138.70, 37.91 ug/m^3, for MTA-New York, PATH-New York, Washington, Boston and Philadelphia, respectively. The average ambient PM2.5 concentration across these cities was 15.91 ug/m^3. Stations serviced by PATH had the highest $PM_{2.5}$ concentrations, as calculated by gravimetric analysis, we had observed in the world, a mean of 1204.25 ug/m^3. A measurement at the Christopher Street station was valued as high as 2773.33 ug/m^3. We also observed significant differences between on-train and on platform and AM and PM $PM_{2.5}$ concentrations. Furthermore, there was considerable variability in OC and EC concentrations between and within cities. Our results show that there is considerable variability in the $PM_{2.5}$ concentrations across cities in the Eastern United States. One train system, the PATH, is particularly polluted with gravimetric measurements calculated at almost 1000 times ambient levels. Means to reduce and to identify the sources of PM in these stations should be undertaken.

2851 Asbestos Exposure Risk from Ceiling and Other Building Materials

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Asbestos exposure risk was assessed from review of bulk samples collected from 27 sites from twelve states. A total of 288 bulk samples contained asbestos. Data was obtained from 18 commercial property sites and 9 residential properties. All asbestos-containing material (ACM) identified in all samples contained Chrysotile. Chrysotile content ranged from 1.25% to 70% among the various bulk samples. A total of 41 samples from popcorn or acoustical ceiling texture material were analyzed. Chrysotile content in popcorn or acoustical ceiling texture ranged from 2% to 10%. The data set contained samples from Florida, California, Colorado and other western and southeastern states. The exposure risk from textured ceiling material including popcorn and acoustical ceilings is associated with disturbance, detachment, and removal of textured surfaces. Disturbance of asbestos- containing building material may result in fiber release and mobilization of asbestos containing materials. Building materials such as duct paper contained the highest 70% ACM and roofing material exterior plaster contained the lowest 1.25% ACM. Also, caulk, wall covering, and other building materials contained asbestos. Eight personal breathing zone air samples were collected following NIOSH Method 7400 during the removal of asbestos containing textured ceiling material under negative pressure in a commercial building in Oklahoma. The personal exposures ranged from below the lab's reporting limit to 0.0201 f/cc. Area air sampling was also conducted during a large-scale (residential) asbestos removal project in Florida. A total of 400 area samples were collected during the removal of asbestos containing materials (textured ceiling, caulk, mastic and drywall) inside an active containment under negative pressure and outside of the containment structure to approximate personal breathing zone exposures. The airborne asbestos concentration ranged from below the labs reporting limit to 0.0135 f/cc. Textured ceiling material and other ACM building materials may increase the risk of asbestos exposure in commercial and residential structures.

2852 Analysis of NIOSH Health Hazard Evaluations Featuring Lead (Pb)

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Lead (Pb) is used today in electronics, smelting, ammunition, and galvanized steel. Given this variety of possible worker exposures, evaluations summarizing occupational exposures are valuable. This analysis of 80 health hazard evaluations (HHE) with data collected by NIOSH from 1991 to 2017 found that work-related Pb exposure data has been collected in at least 32 US states. In addition, HHEs from Washington D.C., Puerto Rico, and Bolivia, were included in the analysis. Many different sectors were covered as defined by the National Occupational Research Agenda (NORA). Four (5 %) of these evaluations were from warehousing and utilities, 5 (6 %) were from public safety or healthcare, 11 (14 %) were from wholesale and retail trade, 12 (15 %) were from construction, 17 (21 %) were from manufacturing, and the majority, or 31 (39 %) were from the services sector. Given the large variety of site-specific needs and types of work covered by these NIOSH evaluations, it is not surprising that programs protecting workers from Pb were not uniformly established. Of these 80 HHE studies, 19 (24 %) reportedly had a respiratory protection program (RPP) and 16 (20 %) had a lead surveillance program (LSP). Ten (13 %) of the HHEs discussed workplaces with both a LSP and a RPP. The HHEs documented Pb exposures in regions across the United States, including Oregon, Colorado, Texas, Wisconsin, Florida, and Maine. OSHA Region 5, or the mid-western states of MN, WI, MI, IL, IN, and OH were the most represented in these HHEs. Twenty-eight percent, or 22 of 80 evaluations, were represented by Region 5 out of the ten possible OSHA regions. A wide range of exposure levels were also observed across this sample of occupational Pb exposures; air concentrations were documented in some situations as not detectable (ND), while the highest sample read 5900 µg/m^3. Blood lead levels in these participants ranged from ND to 40 µg/dL. This analysis demonstrates that occupational Pb exposures are observed in workers across the United States and throughout many different types of industries. This analysis also highlights the need for future collaborations with NIOSH as employers set up respiratory protection plans, safety programs, and medical surveillance to protect the health of workers. The wide variety of settings where Pb exposures can take place demonstrates that Pb remains a priority issue in occupational health within the United States of America. *Disclaimer: The views in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.*

2853 Occupational Biomonitoring and Interpretation of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Using Field Deployable Sensors and PBPK Modeling

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Onsite rapid biomonitoring of occupational exposures supports safe working conditions and practices. Traditional quantification methods of chemicals in biofluids may have long turnaround times leading to delayed analysis and, if warranted, remedial action, highlighting the need for faster measurements and interpretation of measurements. In order to improve measurement and interpretation time of 2,4-dichlorophenoxyacetic acid (2,4-D), we developed a field deployable device for 2,4-D detection and a computational pharmacokinetic model for measurement interpretation. The antibody-based device features an optical-sensing platform that utilizes a smartphone to take measurements of colorimetric changes dependent on the presence of 2,4-D in biological samples. After optimizing conditions, the device detected 2,4-D in the linear range of 1 - 40 µg/L in biofluids. For measurement interpretation, we developed a physiologically based pharmacokinetic model (PBPK) to predict 2,4-D disposition in rats and humans and utilized a Transwell *in vitro* system to parameterize salivary transport of 2,4-D. The PBPK model predicted 2,4-D concentrations in urine (533 µg/L) of 2,4-D backpack applicators following an 8-hour workday exposed to 0.008-0.012 mg/kg/day of 2,4-D. At those same exposure conditions, the model predicted concentrations of 2,4-D in saliva at lower levels than urine (average 1.49 µg/L), but still within the linear range of the sensor. Since these expected levels are well within the detection

limits of the sensor, this device may be suitable for non-invasive and rapid onsite screenings in occupational settings. *Supported by CDC/NIOSH grant R01 OH011023.*

2854 Effects of Occupational Exposure to Low-Level Benzene on Oxidative Damage in Chinese Workers

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Benzene is not only a common environmental pollutant, but also a common organic solvent and chemical raw material in industry and chemical production. Because of the relevant protective measures have been taken by government and enterprises, level of the occupational benzene exposure of most Chinese workers are below the occupational limit (PC-TWA 6mg/m^3). The purpose of this study was to explore the effects of occupational exposure to low-level benzene on oxidative damage of workers. A total of 90 workers exposed to benzene and 85 non-exposed workers were enrolled in this study. The benzene monitoring data in workplace and the health examination data of workers were collected. Blood routine was examined, the S-phenylmercapturic acid (S-PMA), metabolite of benzene in urine, was measured by high performance liquid chromatography-mass spectrometry. The content of lipid peroxide malondialdehyde (MDA) in plasma and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine were detected by colorimetry. The effects of benzene exposure on oxidative damage were analyzed by adjusting sex, age, BMI, smoking and alcohol consumption, and the correlation between S-PMA,8-OHdG and MDA was analyzed. Benzene concentrations at all workplace monitoring sites were below 0.6 mg/m^3. The blood routine results showed that the average red blood cell and platelet counts in the exposed group were significantly lower than those in the control group ($p < 0.001$), but still located within normal limits. The results of S-PMA test showed that average concentration of S-PMA in urine of exposed workers was significantly higher than that of control group (26.54μg/g Cr vs 7.84μg/g Cr, $p < 0.005$); average concentration of MDA in plasma and 8-OHdG in urine of exposed workers were also higher than that of control group ($p < 0.005$); the urine concentration of S-PMA was positively correlated with the 8-OHdG. These results suggested that occupational exposure to low-level benzene still could increase the oxidative damage in workers and the S-PMA, metabolite of benzene had a good correlation with the oxidative damage induced by benzene. *This work was supported by the National Natural Science Foundation of China (Grants no. 81872645, 81730087).*

2855 Direct Quantification Method for S-Phenyl Cysteine Benzene: Oxide Hemoglobin Adduct

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Benzene oxide adducts of hemoglobin have been used as biomarkers of benzene exposure in rodent models and exposed human populations using gas chromatography mass spectrometry methods. This method entails reacting dried protein with trifluoracetic anhydride and methanesulfonic acid to yield phenyltrifluorothioacetate, the ultimate product that is quantified. In this study, we developed a high performance liquid chromatography-isotope dilution mass spectrometry method for the directed quantification of S-Phenyl Cysteine (SPC) , a benzene oxide adduct of hemoglobin cysteine, following acid hydrolysis of globin. The use of electron spray ionization on a triple quadrupole mass spectrometry system was employed to demonstrate a linear response in SPC concentration ranges from 200 fmol to 100 pmol with a 13.51% coefficient of variation between triplicates of samples. This new method showed a sensitivity of 20 pmol SPC g^{-1} globin, similar to the prior gas chromatography method, but requires no derivatization. SPC remained unchanged following hydrolysis and was determined to be stable for direct quantification. Ample precision, accuracy and sensitivity shown in this method makes it ideal for environmental exposure quantification. Validation for environmental exposure is currently in progress using human samples following hurricane related exposures in Houston.

2856 Assessment of Mineralogical Composition and Ecological Risk of Potentially Toxic Metals in Surface Sediments of a Tropical Estuary

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The bioaccumulation in excess of natural loads and potential for remobilization of sediment bound metals in aquatic ecosystems is a major environmental concern. The study assessed the degree of contamination and risk associated with ten heavy metals in forty-five surface sediments from fifteen sites in Lagos Lagoon. Metals concentrations was determined using Agilent 7500c Inductively Coupled Plasma Mass Spectrometry (ICP-MS) while mineralogical composition was examined using X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM). SEM and XRD analyses indicate that alumino-silicate and silty clay minerals influenced metals mobilization and bioavailability from sediment. The result showed that Fe with a mean concentration of 26031.96 mg/kg was the most abundant. The observed metal levels in the order; Fe > Mn > Zn > Cu > Cr > Pb > Co > Ni > As > Cd did not exceed the threshold limits for the protection of aquatic life. Estimated pollution load index (PLI), geoaccumulation index (Igeo), contamination factors and degree of contamination also indicate low to moderate environmental risks. However, the result of enrichment factor demonstrated high level of enrichment from anthropogenic sources which might still pose adverse biological effects. This calls for continuous monitoring and effective legislation in the region.

2857 Biomarker Identification for Human Exposure to Petrogenic Polycyclic Aromatic Hydrocarbons

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On April 20, 2010, the Deepwater Horizon (DWH) oil rig explosion released approximately 4.9 million barrels of crude oil into the Gulf of Mexico. Communities that rely heavily on the Gulf for their livelihood and sustenance were deeply concerned with the long-term health effects of consuming seafood laden with crude oil. Despite extensive studies on the Aryl Hydrocarbon Receptor (AHR)-mediated toxicities of pyrogenic polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene, data on the human health impacts of petrogenic PAHs found in crude oil is scarce. This study aims to identify candidate biomarkers of exposure to petrogenic PAHs that can activate AHR-mediated pathways. Plasma samples were collected longitudinally from 100 volunteers in each of four partner communities (Biloxi, Mississippi; Gulfport, Mississippi; Houma, Louisiana; and Galveston, Texas) between 2012 and 2014. Extensive survey data, health assessments, and blood and urine clinical analyses were also collected. Total plasma PAH levels were measured using Gas Chromatography-Mass Spectrometry (GC-MS) and AHR-mediated activities were quantified using a modified Chemically Activated LUciferase gene eXpression (CALUX) bioassay. Results show that individual total plasma PAH levels did not correspond well with AHR activation, consistent with the presence of distinct bioactive and potentially harmful PAHs in some samples. Of the 42 PAH congeners tested, the petrogenic PAH C3-Naphthalene body burden showed significant positive correlation with AHR bioactivity and may serve as a candidate biomarker. Marked differences in total PAH levels and AHR bioactivities were seen in the subjects from Biloxi and Gulfport despite residing in the same Gulf Coast region. These indicate possible influences from other factors that may be identified using the health assessment and survey data. A general increase in total plasma PAH levels across the 3 years was also observed which may be attributed to an increase in seafood consumption in the ensuing years after the DWH oil spill. This study highlights the need for long-term monitoring of human exposure to sources of crude oil contamination. Future studies include identification and validation of biomarkers of effect to petrogenic PAHs that may aid in risk assessments. *This work is supported by NIH grants R01ES026874, U19ES020676, 1P30ES06676, and T32ES007254.*

2858 Assessment of Lead Exposure Controls on Bridge Painting Projects Using Worker Blood Lead Levels

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A retrospective analysis of worker BLLs was conducted using blood lead data collected by four bridge painting contractors before and after lead exposure. The objective of the study was to evaluate the efficacy of exposure controls in preventing elevated blood lead levels (BLLs) (> 25 µg/dl) during bridge painting projects. The contractors selected for the study submitted BLL data for 289 workers representing ten work tasks and 11 bridge painting projects. In total, 842 BLL results were evaluated. The mean BLL for all workers was 10.8 µg/dl at baseline compared with 14.9 µg/dl after two months of exposure and 15.0 µg/dl after four months of exposure. 28.6 % of the painters and 35.2 % of the laborers had a 10 µg/dl incremental increase or greater in BLL two months after initial exposure. Likewise, 18.4 % of the painters and 25.9 % of the laborers had a BLL greater than 25 µg/dl during the same time. The BLLs that exceeded the 25 µg/dL threshold ranged from 30µg/dL-63 µg/dL for painters and 26 µg-56 µg/dL for laborers. All work tasks with high-intensity exposure (abrasive blaster/painter, abrasive blaster, painter & laborer) experienced an average BLL increase that ranged from 0.24 µg/dl to 8.9 µg/dl two months after initial exposure. In contrast, two months after the initial follow up blood testing, 3 out of the 4 work tasks with high-intensity exposure showed a decrease in average BLLs (range -2.6 µg/dl to -3.1 µg/dl) suggesting the reduction is associated with a modified exposure control. These results indicate the evaluated exposure controls are not effective at maintaining worker blood lead levels in some of the work tasks with high-intensity exposure to the targeted goal of 25 µg/dl. They also suggest that blood lead sampling should be conducted every month after baseline until blood lead levels are controlled to an acceptable concentration.

2859 Delta-9-Tetrahydrocannabinol (THC) Exposure from Vaporized Cannabis Concentrate in Regulated Markets Is Lower Than Smoked Cannabis

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Cannabis concentrate aerosolization ("vaping") is a new and increasingly common method of cannabis consumption. A frequent criticism of cannabis concentrates is their high potency, and the implication that this increases THC exposure-related harm. However, due to the novelty of the consumption method as well as the regulatory challenges inherent in studying cannabis, the literature on THC concentrate inhalation is remarkably sparse. This presents significant challenges for public health officials and policymakers. This study aims to evaluate how delta-9-tetrahydrocannabinol (THC) exposure from aerosolized THC concentrates ("vaping") compares to that from combusted cannabis flower (joint smoking). Real world usage data demonstrate that THC exposure is lower for vaping than joint smoking for the majority of users, even though cannabis concentrates have a much higher THC concentration than cannabis flower (70-90% vs. 10-25%). Usage data from tens of thousands of regulated cannabis users that opted-in to sharing their data were collected via a mobile app connected to a digital vaporizer. Several parameters were measured (including puff timing, frequency, duration and aerosolization energy), and input into a machine-learning model to extrapolate dosages. Data collected from January 2018 through July 2019 show that daily THC exposure via aerosolized cannabis concentrates is significantly lower than exposure from smoking cannabis flower (based on consumption practices cited in the published literature). This is further supported by research showing that cannabis smokers self-titrate their puff size and puff number based on THC potency and expected effects. Consumption of unregulated (illicit) cannabis concentrates is likely higher, as these products are often diluted ("cut"); thus consumers adjust their habits and practices to achieve similar THC exposure and expected psychoactive effects. This model may be used to assess the risks of other toxicants to which consumers may be exposed.

2860 Characterization of Volatile Organic Chemicals (VOCs) in Air Collected on Synthetic Turf Fields in California

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There are more than 900 public or private synthetic turf fields in California. Synthetic turf utilizes a crumb rubber infill made from ground-up recycled waste tires, which have a complex and largely unknown chemical composition. Concern has emerged about the possible health effects of inhalation exposure to chemicals released from synthetic turf fields. Potential exposure to athletes and bystanders of all ages can occur during both sport and non-sport related activities. Diverse environmental conditions and age of the field also likely influence the release of chemicals from the turf. OEHHA sampled 35 fields of various ages across California and is assessing the potential health risks from uses of these fields. We sampled the air off-field and at on-field locations close to soccer activities at 4 heights (4 inches to 65 inches) above field surface. A total of 24 tire-related volatile organic chemicals (VOCs) and 43 common air pollutants were targeted in the air sample analysis using gas chromatography/mass spectrometry. We identified 59 VOCs from air collected on synthetic turf fields. A few tire-related VOCs (benzothiazole, methyl isobutyl ketone, and 2-methylfuran) displayed a height-dependent concentration gradient, with concentrations decreasing with increased height. Many of the common environmental pollutants, xylene, toluene, and phthalates, were present in similar concentrations at the various heights. The chemical concentration gradient noted between tire-related VOCs and common pollutants informs the extent that the synthetic turf field is the source of emission of these tire-related VOCs. This information will assist in assessing human health risks from chemicals released from the synthetic turf.

2861 Risk Assessment of Glyphosate Exposure from Consumer Application of Roundup Using a Margin of Safety Approach

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Due to the widespread application of glyphosate, a non-selective herbicide used in a variety of products, its environmental presence is common such that the general population is exposed to glyphosate in foods and in drinking water. Despite this, environmental exposures to glyphosate are considerably low in comparison to those exposed occupationally. Although previous studies have evaluated exposure to horticultural workers, no study to date has characterized the absorption and biological fate of glyphosate in consumer applicators following the use of glyphosate-containing herbicides in residential yard and garden settings. Therefore, the objective of this study was to measure glyphosate levels in urine over time following consumer application of a commercially available glyphosate-containing herbicide (Roundup®) and estimate the corresponding systemic dose in order to perform a risk assessment using a margin of safety approach. Considering that consumer applicators of glyphosate-containing herbicides are presumably exposed predominantly through inhalation and dermal routes, we evaluated each exposure route separately. In the current study, there were six study participants (i.e., "applicators") per exposure group with equal representation of each gender, for a total of 12 subjects. Each applicator sprayed approximately 16.3 gallons of a 0.96% glyphosate-containing solution (after diluting per manufacturer instructions) for 100 minutes in a gravel yard. Here, we found that, regardless of exposure route, urinary glyphosate levels generally peaked within six hours following application and were statistically indistinguishable from background levels at 24 hours post-application. In general, dermal exposure was greater than inhalation exposure. Moreover, even with the use of conservative assumptions for internal dose estimations, all 12 consumer applicators had internal doses of glyphosate below internal doses estimated from the established health-based guidance values. This pilot study demonstrates that glyphosate exposure from consumer application of a commercially available glyphosate-containing herbicide is not a health concern; however, large-scale studies are necessary to corroborate these findings.

2862 Evaluating Allergic Contact Dermatitis Risk for Organic Residuals Detected in Consumer Products

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Risk of allergic contact dermatitis from consumer products is increasingly becoming a public health concern and consumer product liability risk for manufacturers. This is relevant with the increase of wearable electronic and other wearable consumer products, which can increase the leaching or dermal load of potential sensitizers. Previous research and predictive model development have shown that several well-characterized sensitizers, including nickel, chromium and acrylates, demonstrate a similar log-normal relationship between their likelihood of ACD elicitation and their dermal load. The objective of this assessment was to evaluate the previously described model's potential application to new organic sensitizers. These sensitizers are found as organic residuals in a variety of common polymer and adhesive materials. Patch test data for these organic sensitizers were compiled from regulatory and medical chemistry databases, and then selected based on data quality. The selected patch test data was then evaluated on goodness-of-fit for the previously established log-normal model. Finally, a dermal load (μg/cm^2) was calculated for each sensitizer that was correlated with an approximate 5% level of ACD elicitation risk among sensitized individuals. This 5% elicitation risk is equivalent to the calculated elicitation risk for the current nickel dermal load standard (0.5 μg/cm^2) evaluated under EN 1811 as set by the European Nickel Directive. In summary, we report that common organic sensitizers, found in modern adhesives and polymers, follow a similar relationship as sensitizers that already have regulatory oversight. This relationship can be leveraged along with patch test data from the literature to evaluate the potential elicitation risk for detected organic sensitizer residuals in consumer products.

2863 Implementation of Online Sample Extraction: the Final Step Toward Fully Automated LC-MS Urine Drug Screening

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Systematic toxicological analysis (STA) using comprehensive screening approaches is a major part of everyday work in toxicology, usually involving different analytical techniques. The great benefit of immunoassays (IA) in routine screening of body fluids is the high degree of automation regarding sample preparation and reporting of results. In recent years, LC-MS has become a key technique in STA, but in contrast to IA an appropriate sample preparation is crucial for screening of biological samples. Offline liquid-liquid extraction (LLE), solid phase extraction (SPE) or protein precipitation (PP) are often laborious but mandatory steps and their integration into the analytical workflow is the missing piece towards a fully automated LC-MS screening analysis. The goal of this work was to implement an online μSPE into an existing LC-MS method to achieve a fully automated LC-MS screening of urine samples. Full integration of the online sample extraction and LC-MS screening method was achieved, and developed solution was extensively tested. Recovery Efficiency (RE), Matrix Effects (ME), Limits of Detection (LOD) and reproducible of results have been analyzed and validated with real samples in comparison to routine sample preparation and STA. All μSPE-LC-MS screening results were in good agreement with the initial routine analysis. LC-MS screening of fortified urine using online μSPE led to similar or better results than the routine sample preparation. The online extraction time of approximately 14 minutes fits well into the run-time of LC-MS analysis. Fully automated LC-MS screening eliminates the human factor in the routine analysis and not only improves productivity and reproducible, but also minimizes the exposure of the lab staff to potentially infectious samples.

2864 Estimation of MMP2 and MMP9 in the Plasma of Ill Gulf War Veterans

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Matrix metalloproteinases are essential enzymes that degrade extracellar matrix proteins and are also known to cleave cell surface receptors. These enzymes play a dynamic role in cell maintenance, proliferation, differentiation, tissue remodeling, tissue repair, and in host defense, capable of cleaving cell surface receptors and even inhibiting cytokines under normal conditions. Aberrant activation of these MMPs can release apoptotic ligands, resulting in cellular dysregulation in various systems, including muscles and neurons, and eliciting immune responses, leading to inflammation in some. MMP2 and MMP9 are initially synthesized as inactive zymogens with pro-enzyme domain, which gets removed when the enzyme becomes active. The pro-peptide domain consists of conserved cysteine residue that interacts with zinc in the active site, maintaining an inactive form by preventing the binding and the cleavage of the substrate. Activation of pro-MMP2 and pro-MMP9 are mediated by thrombin, activated protein C and plasminogen activator/plasmin system and the activity is also controlled through TIMP-3. Since MMP2 is gelatinase A and MMP9 is gelatinase B, they are upregulated at sites of tissue damage and inflammation. It can also be activated by several cytokines and growth factors. Increased levels of MMP2 and MMP9 (Matrix metalloproteinase-MMP) are implicated in several diseases including neurological diseases. In this study, we estimated the level of MMP2 and MMP9 in the plasma of 30 Gulf War veterans with Gulf War Illness (GWI), 30 healthy controls (HC), 20 asymptomatic Gulf War veterans (aGWV), and 30 subjects with irritable bowel syndrome (IBS). We performed Enzyme Linked immunosorbent assay and a functional zymography to determine the pro and active MMP2 and MMP9. The active form of MMP2 and MMP9 were significantly increased in GWI compared to other groups ($p<0.0001$) and was increased twofold in IBS subjects when compared to aGWV and HC ($p<0.01$). Not much of a difference was observed between aGWV and HC. *This study was supported by GWIRP award GW170103 - W81XWH-18-1-0454.*

2865 Assessment of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Contamination in Surface Water of Oil Producing Communities in Delta State, Nigeria

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Oil spillage and pollution of surface water bodies is commonplace in oil producing areas in Nigeria. Three major rivers namely; Obosi, Olokun and Abalaka rivers were assessed for their levels of total hydrocarbon (THC). The BTEX measurements in all the rivers exceeded National Drinking Water maximum contaminant levels (MCLs) in 90, 30, 12, and 8% of the samples, respectively. Obosi river had the highest THC values of 972 mg/l. While Olokun and Abalaka rivers recorded THC values of 757.97 mg/l and 402.52 mg/l respectively. Seasonal variation in the concentration of THC in water samples from these rivers showed that there was significant ($P<0.05$) difference in the measured concentration of THC between dry and wet seasons. The concentration of BTEX in these surface water bodies indicated a high level of environmental toxicity. Impact of decades of oil spills in the Niger delta region would have acute and long-term effects on human health. The findings in this study, makes clear indication that the composition and quality of surface water bodies around oil producing areas are being altered by hydrocarbon constituents from oil spillage. The impact of this study was to assess BTEX levels and the environmental impact on biodiversity.

2866 Assessment of Microbial Toxicity Level in Hospital Wastewater in Enugu Metropolis, Nigeria

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The problem of contamination of the environment by hospital and industrial effluent or wastewater has been an issue of concern to the public health of any area where these industries and hospitals are located. This study sets out to determine the level of contamination and toxicity levels caused by the discharge of wastewater from different wards at Kenechukwu Specialist Hospital Thinker's corner, Enugu, Nigeria. The microbial pathogens and toxic metal analyses of the hospital wastewater were determined using established procedures and standards. Wastewater samples were collected from three wastewater outlets of the hospital with pre-cleaned sterile and dried containers. The three sampling points were from Theatre Ward-(TWWS), Maternity ward-(MWWS) and Laboratory ward-(LWWS). The isolated pathogenic bacteria include *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Shigella* sp., Bacteriodes *sp., Citrobacter* sp. and *Enterobacter* while the isolated non-pathogenic organisms were *Pseudomonas aeuroginosa, Serratia* sp. and *Bacillus* subtilis. The result also showed the presence of *Aspergillus flavus*, a pathogenic fungus as the only isolated fungi species in all wastewater samples from the different wards. High level of microbial contamination was observed in wastewater effluent from the maternity ward (MWWS). The toxic metals analysis showed the presence of Arsenic, Lead, Mercury and Chromium. Results shows that toxic metals were within the threshold of WHO permissible limit. Therefore, it was recommended that it would be necessary to properly treat all the hospital wastewater before discharging into the environment to avoid the possible

environmental health risk that might be associated with the discharged contaminated wastewater. Close monitoring of the toxic metal concentration of the discharge water is also suggested.

2867 Determining Potential Sources of Persistent Organic Pollutants in Contaminated Human and Animal Food

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Persistent Organic Pollutants (POPs) present in human and animal food have been a big concern for food safety due to their persistence and toxic effects. To ensure food safety and protect human health from POPs, it is important to understand sources of POPs contamination and then minimize human dietary exposure. In the US Food and Drug Administration (FDA), to determine the sources of POPs contamination, human experts need to manually identify the congener pattern in the mass spectra data of contaminated sample and compare it with potential sources samples results. However, the visual comparison is time consuming and solely dependent on the human expert. To improve the accuracy and reproducibility, we developed a software to assist identifying sources of POPs contamination by quantifying the similarities between the contaminated sample and potential source samples data. Different types of similarities scores were computed and used to rank potential source samples based on user's need. We tested the software on a diverse set of contaminated samples by comparing the list of sources determined using the software with those from human expert analysts. The tests confirmed that the software meets user's requirement and provides results consistent with human experts' observation. In conclusion, the software improved the accuracy and reproducibility of the evaluation process by providing consistent quantifying results and reducing the need for user intervention.

2868 Biosensor Technology Applications in Galveston Bay and Houston Ship Channel: Rapid Analyses for Soils and Sediments

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After Hurricane Harvey, multiple soil and sediment samples were obtained from Manchester, TX, Galveston Bay and the Houston Ship Channel (GB/HSC). Given the suite of organic analytes requested for these samples a rapid and efficient prioritization method was necessary. The purpose of this study was to prioritize samples for exposure risks of polycyclic aromatic hydrocarbons (PAHs). The Virginia Institute of Marine Science (VIMS) biosensor technology was implemented to prioritize PAH analysis for soil and sediment samples. Coupled with gas chromatography/mass spectrometry (GC/MS), individual PAH analysis found alkylated PAHs were dominant within sediments rather than EPA Priority 16 PAHs. Soil samples differed in composition as low molecular weight PAHs and the EPA Priority 16 PAHs were primarily present. Based on these patterns, soil hazard quotients (HQs) were calculated for a child's ingestion risk (range: 1.17E-4 to 6.37E-2). Both of these values are well below 1, where levels exceeding 1 would be considered samples of concern. 2016 sediment cores were analyzed to establish relevant depths to consider in future sampling trips to GB/HSC. The depths containing the highest PAH totals included 30-35cm, 35-40cm, and 40-45cm and both cores primarily consisted of perylene (0.01607-0.52559 mg/kg; 0.01821-0.48393 mg/kg) and pyrene (0.03932-0.16417 mg/kg; 0.09782-0.20832 mg/kg). Initial pore-water analysis streamlined GC/MS analytical workload by prioritizing samples; whereby the GC/MS results contextualized the data for risk characterization. Despite all samples exhibiting low risk, the prioritization and risk screening methods established by this project and a 2019 sampling trip, will afford field scientists to rapidly characterize a site for PAH gradients. *Supported by T32 ES026568; NIEHS P42 ES027704, 3R01ES024245, 2018 KC Donnelly Supplement Award.*

2869 Formulation Development and Validation of an Analytical Method for a Combination Dose Formulation of Abacavir Sulfate, Dolutegravir Sodium, and Lamivudine in Support of Rodent Toxicology Studies

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Treatments for acquired immunodeficiency syndrome generally include combination therapies of antiretroviral drugs. The combination of abacavir sulfate (ABC) sodium dolutegravir (DTG), and lamivudine (3TC) is being used for the treatment of HIV-1 infection in adults. However, there is limited nonclinical safety information, specifically through maternal transfer of the agents to offspring. In this work, a procedure was developed for a combination formulation of ABC/DTG/3TC in 12:1:6 ratio (consistent with the clinical dose ratio) in aqueous 0.2% methylcellulose/0.1% Tween 80 for gavage administration in rodents. An analysis method, using ultra-performance liquid chromatography coupled with photodiode array detector, was developed and validated to determine the concentration of individual drugs in a single chromatographic run over the range 2.4/0.2/1.2 to 72/6.0/36 mg/mL (ABC/DTG/3TC). Validation parameters included linearity ($r \geq 0.9961$), accuracy (% relative error ≤ 14), and precision (% relative standard deviation (RSD) ≤ 8.0). The method was suitable for analysis of formulations with target concentrations up to 130/10.8/65 mg/mL (ABC/DTG/3TC) by diluting in to the validated range with blank vehicle. Homogeneity was successfully demonstrated on formulations containing ABC/DTG/3TC at 3.6/0.3/1.8 mg/mL (low) and 100/8.33/50 (high) with RSD $\leq$ 4.1%. Stability was evaluated at 3.6/0.3/1.8 mg/mL for up to 62 days at ambient and refrigerator temperatures. Formulations were stable when stored at ambient (88 to 104% of Day 0) or refrigerated (91 to 109% of Day 0) temperatures. Formulations were also stable for up to 3 hours under simulated dosing conditions (ambient temperature, exposed to air and light). In conclusion, a formulation and analysis method was developed to quantitate individual drugs in a combination formulation of antiretroviral therapy in support of rodent toxicology studies.

2870 Rare Earth Elements Toxicokinetics after Oral and Intravenous Administration in Rats

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In the past decades, there has been an increasing extraction and use of rare earth elements (REE) in several sectors. However, there is a paucity of data on their levels in the general population and their toxicokinetics. The aim of this study was to determine the biodisposition kinetics of 4 REE (neodymium (Nd), yttrium (Y), praseodymium (Pr) and cerium (Ce)) after a single oral and intravenous exposure in rats. Groups of adult male Sprague-Dawley were exposed by gavage to two different doses (100 and 1000 mg/kg bw) and by intravenous injection to a single dose (1 mg/kg bw) of $PrCl_3$, YCl_3, $NdCl_3$ and $CeCl_3$ (n = 5 per dose and per group of rats). The kinetic time courses of these 4 REE were determined in blood, urine and feces over a period of 7 days following gavage or injection; rats were sacrificed on day 7 post-dosing and tissue levels were measured. Biological levels were determined by ICP-MS. Oral results indicated that these compounds were very poorly absorbed through the gastrointestinal tract, with levels near the analytical limit of detection in blood and tissues. Urinary levels of orally administered dose were very low, with maximum percentages amounting to 0.8% when looking at individual excretion during the 0-72 h postdosing. The fecal profiles in orally exposed rats were similar for all 4 metals and indicated that the majority of the administered dose (both doses) was recovered in feces over the 72-h collection period. The mean (± SD) percentages of administered dose recovered in feces as Pr, Ce, Nd and Y were calculated to be 115 ± 13.6, 101 ± 13.5, 104 ± 22.5 and 87.5 ± 4.9% for the 100 mg/kg dose and 90.2 ± 21.8, 90.3 ± 7.0, 53.2 ± 14.8 and 67.5 ± 14.2% for the 1000 mg/kg dose during the 0-72 h postdosing. The intravenous results showed a similar rapid elimination from blood, with highest levels at 5 min post-dosing and a half-life of on average ≈ 3 h for the 4 metals. A significant tissue distribution was observed with the highest concentrations found in the liver (33.7 ± 16.8, 49.3 ± 12.3, 37.4 ± 17.5 and 10.3 ± 11.8%, respectively for $PrCl_3$, $CeCl_3$, $NdCl_3$ and YCl_3) followed in decreasing order by the spleen, kidney and lungs. Urinary excretion following intravenous injection was very low. However, fecal profiles were similar for all 4 metals and showed that excretion was not complete 7-day postdosing, asymptotic values not being reached at that time point; the mean (± SD) percentages of injected dose recovered as Pr, Ce, Nd and Y were respectively 19.2 ± 8.7, 26.7 ± 5.3, 22.1 ± 3.8 and 42.8 ± 17.6% during the 0-7-day period postdosing.

2871 Disposition of Microcystin LR in Rodents: A Potential Explanation for the Limited Toxicity Observed in Rodents following Oral Exposure

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Microcystins constitute a large class of monocyclic heptapeptides and are the most commonly found cyanotoxins worldwide. Exposure to microcystins can occur via ingestion of contaminated water and food or by inhalation of and dermal contact with contaminated water. Limited toxicity was observed in a recent study by the National Toxicology Program (NTP) following perinatal gavage exposure of CD1 mice up to 1000 µg/kg microcystin LR (MCLR). MCLR was not detected in the plasma of these animals at or above the limit of quantitation (LOQ, 1 ng/mL) of the assay. To help explain the toxicology data and to investigate species difference in disposition, we conducted studies in female CD1 mice and Hsd:Sprague Dawley® SD® (SD) rats following 5d gavage (500 µg/kg) and a single intravenous (IV, 10 µg/kg) administration, and sacrificed up to 24 h after the last dose. In mice, following gavage exposure, parent MCLR (free) in plasma was ≤LOQ of the assay. Of the administered dose, 42% was recovered in feces 24 h after the last dose with low levels in urine (~0.1%); levels in the gastrointestinal tract tissues, liver, and kidney were below the LOQ of the assay. Dose recovered as total MCLR (combined free and bound MCLR and metabolites) was 49% with the majority in feces and low levels (≤0.2%) in urine, liver, and kidney. The levels in the liver didn't change over time from 0.5 to 24 h suggesting that it was covalently bound. In rats, the pattern of disposition following gavage exposure was similar to mice based on free with 36% of the dose recovered; the recovery as total was 94% with the majority in feces and low levels (≤0.2%) in urine, liver, and kidney. Free or total MCLR was not detected in bile in rats. Following IV exposure, in mice, free or total was not detected in plasma at the earliest collection timepoint of 2 h. The dose recovered as free in aforementioned matrices was 1% and as total was 53% (liver, 48%; urine, 4%; feces, 1%). Following IV exposure in rats, low levels of free and total (≤14 ng/mL) was detected in plasma at the first collection time point of 5 min. In rats, the recovery of free was ~ 0.01% and total was 75% (liver, 64%; urine, 4%; feces, 6%). In conclusion, MCLR was very poorly absorbed following oral exposure in rodents and likely retained in the liver leading to poor systemic exposure which may in part explain the limited toxicity observed in NTP toxicology studies.

2872 Disposition and Kinetics of 2,4,6-Tribromophenol in Lactating Rats and Postnatal Day 12 Nursing Pups

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2,4,6-Tribromophenol (TBP, CAS No. 118-79-6) is a widely used brominated chemical used as a flame retardant (BFR), precursor chemical, and wood antifungal agent. Due to a variety of natural and anthropogenic sources, TBP is detected in environmental matrices and biota, including human breast milk, placenta, and serum. In non-occupational exposures, serum levels of TBP correlated positively with those of polybrominated diphenyl ethers, suggesting similar sources of exposure. In addition to potential oral or dermal exposure through dust, TBP is likely to be consumed in a diet rich in wild-caught fish. To address the observations of TBP accumulation in human breast milk, studies were conducted to characterize the disposition and toxicokinetic profile of TBP in nursing Sprague Dawley rats following single oral bolus administration to the dam. Female Sprague Dawley rats (N=4-5/dose group, 14 weeks old) were used in these studies. Litters were culled and balanced to 4 males and 4 females at 4 days postpartum. Animals were maintained in an AAALAC-approved animal care facility. Dams were administered a single dose of [^{14}C]-labeled TBP by gavage on postnatal day 12 (10 µmol/kg, 25 µCi/kg, 4 mL/kg) and pups were allowed to nurse freely. Following administration of the compound, animals were euthanized between 0.25 and 24 h post dose and tissues were collected from the dam and nursing pups. Samples were analyzed in parallel for quantitative and qualitative analyses. Dams had a maximum systemic concentration (C_{max}) at 30 min (13 nmol-eq/mL) and fell through 24 h (0.2 nmol-eq/mL). In the dams, peak tissue concentrations were observed between 0.5 & 1 h post-dose where liver, kidney, and mammary tissues contained 3% (7 nmol-eq/g), 1% (20 nmol-eq/g), and 0.3% (3 nmol-eq/g) of the administered dose, respectively. The stomach contents of nursing pups increased over time, with concentrations of ~1 nmol-eq/g observed at 6 and 12 h post-dose, indicative of sustained exposure via milk. [^{14}C]-concentrations were low in pup blood and liver (<0.1 nmol-eq/g) at all time-points evaluated. Concentrations of TBP and its metabolites will be assessed in pup kidney and intestinal contents to determine the route of elimination of milk-ingested TBP. *This research was supported by the NIH Intramural Research Program [ZIA BC 011476].*

2873 Novel Mouse Lines with Humanized Glutathione *S*-Transferase Pi, Mu, and Theta Families Reveal Species Differences in Enzyme Expression and Function *In Vivo*

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Genetic contributions to inter-individual differences in the response to a specific toxic or therapeutic substance are studied in an attempt to personalize or predict individual risk or response to a compound. The glutathione *S*-transferase (GST) enzymes, which facilitate the excretion of ubiquitous endogenous and exogenous electrophilic toxicants, have been extensively studied for their potential contributions to these gene-environment interactions, and the high frequency null alleles in the *GSTM1* and *GSTT1* genes have been linked to altered drug metabolism and increased disease risk. While experimental animal models have been used to further our mechanistic understanding of GSTs, extrapolating the results of an animal study to the human population is complicated by inter-species differences in the number and structure of these genes. Thus, although rodent models have identified a role for GSTs in metabolism, it has often been difficult to assign a specific GST or subset of GSTs to the metabolism of a compound or drug and even more so to determine how this metabolism is affected by common variants in these enzymes. To address this problem, we generated a panel of mice that express the human GST isoforms by deleting the loci carrying the 14 mouse *Gstt*, *Gstm*, and *Gstp* genes and using syntenic replacement to repopulate each locus with the corresponding segment of human DNA. These lines show quantitative and qualitative differences in the expression of the human and mouse *GSTP*, *GSTM* and *GSTP* genes. Furthermore, they reveal species-specific differences in gene induction in response to transcription factor inducers. Using the dietary toxicant acrylamide as a model substrate, we demonstrate species-specific differences in the role of GSTs in acrylamide metabolism and their ability to protect against organ damage. These humanized mouse lines provide a platform for identifying human GSTs that contribute to drug/toxin metabolism and for assessing risk imparted by common disease-associated GST polymorphisms.

2874 Prediction of Drug Hepatic Clearance Using Long-Term Sandwich-Cultured Human Hepatocytes

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Predicting the *in vivo* pharmacokinetic characteristic of new compounds from *in vitro* studies plays an important role in the drug discovery and development processes. The prediction provides essential information for the assessment of drug-drug interaction and time dependent effects and/or toxic events. When a compound reaches the liver via the systemic circulation, hepatic elimination starts with the penetration of the drug through the sinusoidal membrane, followed by metabolism and biliary excretion. In the present project we assessed prediction of hepatic clearance using primary human hepatocytes in a sandwich configuration allowing long-term cultures, *i.e.* by renewing Matrigel overlay until bile canaliculi formation (~6-7 days after seeding), a system in which transporter and metabolic functions have been shown to be maintained [(1,2,3)], and thereafter incubated hepatocyte monolayers for a maximum of 7 days with test compounds. A total of 28 test compounds covering a large range of physicochemical and pharmacokinetic properties were considered in the study. Conventional *in vitro*/*in vivo* Extrapolation (IVIVE) methods based on the Well Stirred model were used to extrapolate the clearance 1. without taking into account any binding to proteins (*i.e.* the so-called direct method) and 2. with taking into account the concentration of albumin produced in the culture medium over the duration of incubation as recently proposed [(4)] (*i.e.* the so-called PLR adjusted method). Our result demonstrated that using the direct scaling method, 71 % of the compounds were well-predicted, within the 2/4-fold envelop (43 % in the 2-fold envelop, 25 % in the 3-fold envelop and 4 % in the 4-fold envelop). Using the PLR-adjusted method, 79 % of the compounds were also well-predicted (43 % in the 2-fold envelop, 25 % in the 3-fold envelop and 11 % in 4-fold envelop). The slowly cleared compounds, *i.e.* the 19 compounds with clearance less than 30 % of hepatic blood flow, were also well predicted, 74% in the 2/4-fold envelop with the PLR adjusted method. Our study demonstrated that by using this relatively simple and easy overlay method for prolonged hepatocyte incubation time, accurate *in vivo* clearance predictions were obtained, especially for compounds with low metabolic clearance. 1. Parmentier et al. (2013) *Drug Metab Dispos*. 41:1835-42. 2. Oorts et al. (2018) *Journal of Pharmacological and Toxicological Methods* 90: 31-38 3. Parmentier et al. (2017) *Arch. Toxicol.* 91: 2879-2893 4. Da Silva et al. (2018) *Journal of Pharmaceutical Sciences* 107:1957-1972

2875 Expression and Immunolocalization of Xenobiotic Transporters at the Human Blood-Testis Barrier

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The blood-testis barrier (BTB) is formed by basal tight junctions between adjacent Sertoli cells of the seminiferous tubules and functions as a physical barrier to protect developing germ cells in the adluminal compartment from reproductive toxicants. Multiple xenobiotics are known to cross into the male genital tract (MGT) including male contraceptives, cancer chemotherapeutics, and environmental toxicants, although the mechanism(s) for their movement into the MGT is(are) largely unknown. Previous studies showed that several ATP-binding cassette (ABC) and solute carrier (SLC) superfamily transporters are expressed in the testes and could potentially facilitate the disposition of xenobiotics into the MGT through concurrent uptake and efflux mechanisms. This study aimed to determine the expression and localization of xenobiotic transporters that may facilitate transepithelial transport across the human BTB. We used immunofluorescence staining to determine the cellular localization of these transporters in the several cell types found in human testes. OAT1, OCTN1, and MRP3 were primarily localized to the basal membrane of Sertoli cells, whereas CNT2, OCTN2, MRP6, and MRP7 were localized to both the basal membrane of Sertoli cells and the peritubular myoid cells that surround the basal membrane of Sertoli cells. Interestingly, OCT1, OCT2, and OCT3 were localized to the peritubular myoid cells and the Leydig cells, although there was minor staining of germ cell nuclear membranes. However, definitive staining of CNT1, OAT2, OAT3, MATE1, MATE2, OATP1A2, OATP1B1, OATP1B3, OATP1C1, OATP2A1, OATP2B1, OATP3A1, OATP4A1, and OATP6A1 was not observed due to non-specific antibody staining or an absence of staining. The localization of CNT2, OAT1, OCT1, OCT2, OCT3, OCTN1, OCTN2, MRP6, and MRP7 suggests these transporters may contribute to the mechanism(s) by which xenobiotics can cross the BTB. Taken together, the localization of these pharmacologically relevant transporters provides insight into the selectivity of drug disposition across the BTB and may be useful in developing tools to understand and overcome the pharmacokinetic and pharmacodynamic difficulties presented by the BTB.

2876 Modeling Blood-Testis Barrier Characteristics with Novel CRISPR/Cas9 Functional ENT1 and ENT2 Knockout HeLa Cell Lines

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Equilibrative nucleoside transporters (ENTs) transport nucleosides across the blood-testis barrier. ENT1 is located on the basal membrane while ENT2 is located on the apical membrane of human Sertoli cells, creating a transepithelial transport pathway across the blood-testis barrier. Improving drug disposition to the testis with drugs that use transepithlelial transport pathways may more effectively treat viral infections and cancer within the male genital tract. Here, we characterized uridine transport in two novel ENT cell lines and assessed the impact of nucleoside reverse transcriptase inhibitors (NRTIs) on uridine uptake to gain further insight on ENT function and substrate selectivity. ENT1 or ENT2 functional knockout HeLa cell lines were generated using CRISPR/Cas9 to determine the kinetics and selectivity of each ENT individually. We demonstrate the functional loss of each transporter through quantitation of [^{3}H]uridine uptake in the presence of the ENT specific inhibitor S-(4-nitrobenzyl)-6-thioinosine (NBMPR). At low concentrations (100nM), NBMPR inhibits ENT1; conversely, at high concentrations (100μM) NBMPR inhibits both ENT1 and ENT2. In ENT2 knockouts, >97% of [^{3}H]uridine uptake was inhibited by 100nM NBMPR, indicating ENT1 function is predominant. In ENT1 knockouts, only 5% of [^{3}H]uridine uptake was inhibited by 100nM NBMPR and 90% was inhibited by 100μM NBMPR, indicating ENT2 function is predominant. The J_{max} for ENT1 is 23.4 pmol cm^{-2} min^{-1} and for ENT2 is 34 pmol cm^{-2} min^{-1}. The K_t for ENT1 is 10μM and for ENT2 is 110μM. We next evaluated the impact of nine NRTIs (abacavir, entecavir, zidovudine, tenofovir, lamivudine, emtricitabine, zalcitabine, and stavudine) on [^{3}H]uridine uptake, revealing abacavir as the most potent inhibitor of [^{3}H]uridine uptake, with an IC_{50} of 72μM for ENT1 and 350μM for ENT2. This suggests that abacavir has higher affinity for ENT1 than ENT2. These functional knockout cell lines will allow further screening of therapeutic compounds to establish the substrate selectivity between ENT1 and ENT2, which can aid in the development of future compounds that are able to circumvent the blood-testis barrier via the ENT1-ENT2 transepithelial transport pathway.

2877 *In Vitro* Biokinetic Input of Organic Anion Transporting Polypeptide (OATP) 1B1 for Prediction of Biliary Excretion

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Application of physiologically based kinetic (PBK) modelling in quantitative *in vitro in vivo* extrapolation (QIVIVE) in human safety assessment is still in a developmental stage as far as use in regulatory frameworks is concerned. Difficulties appear to occur especially for compounds that are eliminated from the body via active excretion in either urine or bile, because *in vitro* models to quantify kinetics for this active excretion remain to be defined and validated. The aim of the present study was to develop a rat PBK model that includes biliary excretion, using estradiol-17β glucuronide (E217βG) as the model compound. E217βG is known to be preliminary excreted in bile via the organic anion transporting polypeptide (OATP) 1B1. Values for the PBK model parameters Vmax and Km required to describe OATP1B1 mediated transport of E217βG were found in literature in six different studies. These studies all used human embryonic kidney HEK-293 cells overexpressing OATP1B1 and a mock transfected cell line to obtain these kinetic parameters. Where the Km values reported were all comparable amounting to 6.3 μM - 12.85 μM, the Vmax values varied substantially between the studies, varying from 1.37 pmol/mg protein/min to 798 pmol/mg protein/min. These Vmax values were scaled to the rat liver taking into account the amount of liver protein and transporter expression in HEK-293 cells as compared to liver tissue. Use of the Km and Vmax values thus obtained in the PBK model, resulted in well-predicted time-dependent blood concentrations and cumulative biliary excretion for an intravenous dose of 81 ng/kg bw for four out of the six parameters sets. The study provides a proof of principle for how biliary excretion can be included in rat PBK models using an *in vitro* cell line to define the kinetic parameters that describe the biliary excretion thereby providing a fruitful approach in alternatives to animal testing.

2878 Altered Cisplatin Pharmacokinetics during Nonalcoholic Steatohepatitis Contributes to Reduced Nephrotoxicity

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Cisplatin is an alkylating antineoplastic agent that is indicated for the treatment of solid malignancies. Cisplatin is preferentially eliminated from systemic circulation via tubular secretion, whereby it exhibits dose-limiting nephrotoxicity. Interindividual variability in xenobiotic transporter expression is a known contributor to differential cisplatin toxicity and efficacy, and may be the result of genetic, environmental, and pathological contributions. In this study, we aimed to determine if nonalcoholic steatohepatitis (NASH) alters cisplatin pharmacokinetics and if this change elicits differential nephrotoxicity. Sprague Dawley rats fed a control or methionine and choline deficient (MCD) diet to model NASH were given a single bolus dose of cisplatin and sacrificed after 72 h. The MCD diet resulted in a NASH hepatic phenotype that remained unchanged following cisplatin exposure. Drug-naïve NASH rats also displayed no evidence of differential renal pathology relative to control rats. However, renal necrosis and inflammation were reduced in NASH by 40 and 63% following cisplatin treatment, respectively, relative to healthy controls. Furthermore, kidney weights of cisplatin-treated control rats were increased by 31%, compared to an 18% increase in NASH. Plasma cisplatin clearance was reduced from 6.78 (control) to 4.04 mL/min in NASH, and cisplatin plasma AUC was significantly increased by 44% in NASH, relative to control. Cumulative urinary elimination of cisplatin was decreased from 73 to 34% of total dose and renal clearance was reduced from 4.64 to 1.49 mL/min in NASH, compared to control. Subsequently, renal intracellular accumulation of cisplatin after 6 h was reduced by 34% in NASH, relative to control. Supporting these findings, expression of proximal tubule cisplatin uptake transporters, Ctr1 and Ctr2, were reduced by 24 and 64%, respectively compared to healthy control rats, whereas expression of Oct1, Oct2, and Oct3 were unchanged. Interestingly, expression of cisplatin efflux transporters Mate1 and Atp7a were reduced by 52 and 31%, respectively, in NASH compared to control. Taken together, these data suggest that NASH alters renal uptake and efflux transporter expression, thereby attenuating cisplatin uptake and clearance in the kidney with a corresponding reduction in renal cell exposure and nephrotoxicity during NASH. As such, this study demonstrates that NASH can influence pharmacokinetics of renally-eliminated drugs which may contribute to adverse drug reactions.

2879 Differential Portal Vein versus Lymphatic Uptake of the Pyrethroids Deltamethrin (DLM) and *cis*-Permethrin (CPM) in Rats

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Little information is available on the gastrointestinal (GI) absorption pathways for the widely used pyrethroid insecticides. GI absorption typically occurs via the portal venous blood (going directly to the liver) and/or the mesenteric lymph. Absorption by one or the other pathway can significantly influence the amount of compound reaching the systemic circulation, as well as sites of tissue distribution and metabolism. Compounds entering the portal circulation are subject to first-pass hepatic uptake and metabolism, thereby reducing the quantity of pyrethroids entering the arterial circulation. Generally, absorption of highly lipophilic compounds or drugs with log P values greater than 5, such as DDT and hexachlorobenzene, occurs through lymphatic vessels. The lymphatic contribution varies depending on the fat content in diet and/or the use of different vehicles. Given the high lipophilicity of pyrethroids, with reported log P values ranging from 5.4 to 6.2 for DLM and 6.5 for CPM, a significant contribution of lymphatic absorption is likely for these two compounds. These studies employed anesthetized adult male Sprague-Dawley rats, in which the hepatic portal vein was cannulated (to collect blood), the duodenum was cannulated (to administer dose compound) and the mesenteric lymph duct was cannulated (to collect lymph). The method required careful surgical technique and a success rate for collection of lymph varied from 50-70% of the animals entering the study. Lymph and portal blood were collected pre-dosing and at various time points up to 300 minutes post-gavage dosing of either 5 mg/kg DLM or 60 mg/kg CPM in a corn oil vehicle (5 ml/kg body weight) and were analyzed for levels of either DLM or CPM. The data demonstrate that the majority of both pyrethroids are absorbed via the lymphatics with 100-1000-fold less being absorbed via portal blood for both DLM (n=5) and CPM (n=4). Additional studies are being performed with both DLM and CPM using the more polar solvent glycerol formal as the dosing vehicle. (Supported by CAPHRA).

2880 Disposition and Kinetics of 2,4,6-Tribromophenol in Pregnant Rats at Gestation Days 12 and 20

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2,4,6-Tribromophenol (TBP) is a chemical commonly used as a wood preservative and a fungicide. It is also used as an intermediate in brominated flame-retardant (BFR) synthesis and is a degradation product of many of these compounds. Even though it originates from a variety of anthropogenic processes, TBP is also a naturally occurring substance in marine ecosystems, serving as a defense mechanism for a variety of organisms therein. Given its widespread use, TBP can be found throughout the environment (soil, marine organisms, mammals, birds), including in human blood, breast milk, and placental tissue. We dosed timed-pregnant HSD:Sprague Dawley rats at gestation day 12 (GD12) and 20 (GD20) with ^{14}C-radiolabeled TBP in a corn oil vehicle (10 µmol/kg, 25 µCi/kg, 4 mL/kg). We euthanized the animals following dosing at 8 different time points (15, 30 min, 1, 2, 3, 6, 12, or 24 h) and harvested tissues (whole-blood, plasma, and organ homogenates). We then oxidized tissue aliquots (~25 mg) and quantified radioactivity using a liquid scintillation spectrometer (LSC). At both gestation ages, the maximal plasma concentrations of [^{14}C]-radioactivity were observed at 15 minutes post-treatment. Plasma C_{max} was 11 nmol-eq/mL in both GD12 and GD20 rats. Plasma concentrations then steadily declined at both GE12 and 20 through 24 h, and distribution to tissues appeared to follow plasma concentrations. [^{14}C]-Radioactivity concentrations in kidneys from GD12 rats rapidly declined after 15 min (21 nmol-eq/g), while kidney concentrations in GD20 rats had a C_{max} of approx. 20 nmol-eq/g at 30 minutes, suggesting that there may be delayed clearance from the kidneys at this late stage of gestation. Furthermore, [^{14}C] in thyroid glands was notably higher in GD20 rats than in GD12 rats during the first 2 hours after exposure, suggesting that the thyroid may accumulate TBP toward the end of pregnancy. Evaluations of TBP disposition in GD12 embryos and GD20 fetuses and their associated placentae are ongoing. *This research was supported by the NIH Intramural Research Program [ZIA BC 011476].*

2881 Developing Toxicity and Pharmacokinetic Models for an *In Vitro* Integrated Organ Platform (HuDMOP)

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In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate (simulated blood flow). This arrangement allows integration of absorption, metabolism and toxicity data over extended times *in vitro* and provides a novel, animal-free tool for chemical, cosmetic, and pharmaceutical testing. In order to test this, a study on the uptake and distribution of acetaminophen (APAP) in a human dynamic multi-organ plate (HuDMOP™) with three tissue surrogates arranged in series: first absorption across a human 3D intestine (EpiIntestinal™, MatTek Corp), then on to a liver surrogate with human primary hepatocytes in sandwich culture and then to a kidney preparation (human renal proximal tubule cells) was developed. A common perfusate with human albumin connected the three compartments. APAP was placed on the apical side of the intestinal surrogate at 0 and 24 hr. Samples were collected from all three compartments over time and analyzed for APAP by LC/MS/MS and cytotoxicity by LDH leakage. The APAP in the uptake reservoir peaked to 60.7 µM at around 4 hours with a total uptake of 72% of the applied dose entering the first reservoir. A simple PK model was developed to describe the three cellular platforms and their physical arrangement. Mass balance equations were fit to experimental data to estimate uptake and transport characteristics. The inter-chamber flow rates and fitted experimental absorption rate constant, 0.79/hr, were consistent with a C_{max} of 62.0 µM and time of maximum concentration between 3 hr and 4 hr in the intestine compartment. With the current platform flow rates, much lower concentrations were present in the subsequent two compartments (liver and kidney) with maximum observed concentrations of 4.5 µM and 2.5 µM versus 3.1 µM and 0.9 µM predicted. The interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in-life behavior of various chemicals.

2882 Structural and Functional Pharmacokinetic Analogs for Physiologically Based Pharmacokinetic (PBPK) Model Evaluation

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Physiologically based pharmacokinetic (PBPK) models enable simulations of absorption, distribution, metabolism, and elimination of chemicals from the body. Model evaluation is a key step in the PBPK model development processes whereby model predictions are compared to pharmacokinetic (PK) data. A prerequisite for PBPK model evaluation has always been the availability of PK data for the modeled compound, a requirement which has limited the use and acceptance of PBPK models since PK data is often limited or not available. The current work tests the hypothesis that an adequately developed PBPK model for a target chemical (chemical with no PK data) can be evaluated using PK data from a source chemical (chemical with existing PK data). Two different approaches for identifying the source chemical, a structural PK analog and functional PK analog technique, are used to evaluate a series of oral human PBPK models for approximately 100 chemical pairs. The maximum concentration in plasma (Cmax) and area under the curve (AUC) predicted by the PBPK model for a target chemical was generally within a factor of three to the experimental data from the source chemical. Results show that both analog approaches can identify PK analogs which display similar PK as the target chemical and can be used as alternative ways for evaluating PBPK models. As animal free safety assessment strategies continue to develop, it's important to develop alternative approaches for PBPK model evaluation which does not rely on generating new PK data.

2883 A Combination of a Physiologically-Based Pharmacokinetic (PBPK) Model for the Inhalational Route and an *Ex Vivo* Model for Prediction of Lung Absorption Kinetics

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Information on systemic available concentrations is important for the safety assessment of chemicals and the pre-clinical development of new drugs. In recent years, the inhalational route has gained particular interest. A Physiologically-Based Pharmaco-Kinetic (PBPK) model for airborne sub-

stances with focus on the uptake via the lung has been developed. The resulting IVIVE-Lung-PBPK model *(in vitro* to *in vivo* extrapolation) shall be applicable to gases, liquid aerosols and (slowly) soluble particles. The lung is divided into three sub-compartments to account for the different clearance and uptake processes in the individual lung sections. The systemic part of the model is based on a state-of-the-art design and includes the different relevant organs/tissues. For uptake through the lung epithelium, permeation values are derived using *in vitro* and *ex vivo* models, like the Isolated Perfused Rat Lung. Further relevant data and processes, such as mucociliary clearance or blood-tissue distribution coefficients are included based on literature data. To investigate the applicability of the model, pulmonary permeability coefficients were determined for ciprofloxacin and a second small molecule substance, for which many further ADME parameter are available from literature. Comparison of the data derived from the PBPK model with the permeability coefficients from the IPL as input parameters to the corresponding human data shows good agreement for the plasma concentration profile and the quantitative concentration levels. According to these investigations, the inhalation PBPK model in combination with the pulmonary absorption parameters determined in the *ex vivo* model of the Isolated Perfused Rat Lung can successfully predict the systemic uptake in humans for small molecule substances with diffusion-controlled transport mechanisms. Therefore, this model can well contribute to the safety assessment of chemicals with usually very limited availability of *in vivo* PK data, as well as for pre-clinical studies with inhalable drugs. Currently, the lung PBPK model is further improved in the project Cefic-LRI B21. This includes a more detailed description of different clearance processes, such as macrophage-mediated clearance and dissolution as well as the investigation of the applicability of other biological parameters determined *in vitro*.

2884 Physiologically Based Pharmacokinetic Modeling to Identify Upper Bound No Effect Thresholds for Human Tracheal Sensory Irritation

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Exposure to sensory irritants may stimulate the trigeminal nerve receptors, eliciting sensory irritation of the eyes/nose/throat and coughing. Recent studies of human volunteers exposed to formaldehyde (FA) provide refined dose-response findings for eye and upper respiratory irritant responses controlled for negative affectivity and FA was utilized as the index compound for modeling dose-response of four other known sensory irritants: acetic acid (AA), diacetyl (DA), peroxyacetic acid (PAA), and hydrogen peroxide (H_2O_2). We applied a computational fluid dynamics/physiologically based pharmacokinetic (CFD/PBPK) model to each of these compounds to estimate no observable effect level (NOEL) thresholds for anterior tracheal sensory irritation by internal dosimetry methods. The 50% decrease in respiratory rate in mice (RD50) for each compound was used as a sensitive measure of relative potency to induce sensory irritation. The anterior tracheal epithelium was considered the primary target tissue for coughing sensation responses, and the model was run assuming no metabolism to assure upper bound estimates for a 15-min exposure duration. The human NOEL identified for FA (0.8 ppm) was used to estimate FA deposition concentration NOELs (mmol/L) in the anterior tracheal epithelium. This FA deposition NOEL was then multiplied by the RD50 ratio (e.g. AA/FA) to adjust for relative potency to estimate the molar deposition NOELs for AA, DA, PAA and H_2O_2. The model was then utilized to identify the airborne concentration of AA, PAA, DA, and H_2O_2 corresponding to the anterior trachea deposition NOEL in a human performing light exercise. Using this method and the well-defined NOEL for airborne FA controlled for negative affectivity bias, the corresponding airborne NOEL concentrations were determined for AA (61 ppm), DA (1000 ppm), PAA (1.3 ppm) and H_2O_2 (25 ppm). These model-predicted NOEL concentrations are higher than occupational standards designated based largely on animal dose-response data for upper respiratory irritation. This approach estimates an upper bound airborne NOEL value since it does not account for rapid metabolism of these example compounds, assumes both nose and mouth breathing, and may be useful for similarly assessing other sensory irritant compounds with RD50 values.

2885 Use of Toxicokinetic Data for Afidopyropen to Determine the Dose Levels in Developmental Toxicity Studies

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Afidopyropen (AF) is an insecticide that acts as a TRPV channel modulator in chordotonal organs of target insects and has been assessed for a wide range of toxicity endpoints including developmental toxicity in rats and rabbits. The AF GLP definitive developmental toxicity study in rabbits did not produce evidence of maternal or fetal toxicity at the highest dose tested (32 mg/kg/day; NOAEL) but pharmacokinetics (PK) in pregnant rabbits exhibited onset of PK nonlinearity, as measured by plasma C_{max} and AUC, at 15, 32 and 60 mg/kg/day relative to 5 mg/kg/day. Following single gavage doses, respective C_{max} and AUC values were: C_{max}: 15.6 ± 17.3, 438 ± 23.8, 2780 ± 498, 4810 ± 721 ng/mL; AUC: 12.6 ± 9.22, 600 ± 193, 6110 ± 752, 24100 ± 3930 hr*ng/mL. The developmental NOAEL of 32 mg/kg/day is 9000X higher than the maximum expected human dietary exposures to AF; the dose range where onset of nonlinear PK was observed, 5-15 mg/kg/day, is 1400 - 4200X higher than expected human exposures. As PK nonlinearity occurred between 5 and 15 mg/kg/day, 32 mg/kg/day is a sufficiently high dose (kinetically derived maximum dose) for a prenatal developmental toxicity study. Regulatory toxicity testing dose selection guidance recognizes onset of saturated PK as evidence of excessive biological stress to test animals when well separated from human exposures, and that any effects noted at such doses have questionable relevance for human hazard identification and risk. In addition, although weak AF dopamine D2 receptor agonist activity is responsible for tumorigenicity in rats only at high metabolically saturating doses, the potent D2 agonists bromocryptine, cabergoline and pergolide mesylate are not teratogenic in rabbits, rats or mice. The *in vitro* AF concentration eliciting a 50% D2 receptor activation (594 ng/ml) is less than the C_{max} at the saturated 32 mg/kg/day AF dose, suggesting that the top rabbit NOAEL dose also presented potential pharmacological stress. Taken together, the current data demonstrate that consideration of PK is critical for improving the dose-selection in developmental toxicity studies to enhance human relevance of animal toxicity studies.

2886 Toxicokinetics of Constituents in *Ginkgo biloba* Extract Administered to Rats

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Investigating the toxicokinetics (TK) of the many constituents in an herbal product provides a complex investigational challenge. In this study, untargeted reverse phase ultra-high performance liquid chromatography-mass spectrometry analyses were used to generate TK profiles of the constituents of *Ginkgo biloba* extract and the data were compared with those generated using conventional targeted analysis methods. Plasma samples (3 per time point) were obtained at 0 (pre-dose) and up to 1440 min after dosing, from F344/NCrl rats administered a single 300 mg/kg dose of *Ginkgo biloba* extract by gavage and analyzed to determine their metabolic profile. Orthogonal Partial Least Squares Discriminant Analysis was used to identify the features (retention time and mass for an analyte) that differentiated between groups at 90, 480 and 1440 min, that were later identified. In plasma, bilobalide, and ginkgolides A, B, C, and J were readily detected, but isorhamnetin, quercetin, and kaempferol were not. However, conjugates of isorhamnetin glucuronide, kaempferol glucuronide and sulfate, and methylated quercetin glucuronide sulfate/isorhamnetin glucuronide sulfate were identified. Several substituted benzoic acid derivatives were also detected. Data for the top 12 constituents (peak area) were analyzed by a noncompartmental method to generate TK data. For a limited set of standards: bilobalide, and ginkgolides A, B, C, and J, quantitative analysis was conducted using a limited six-point calibration curve, and a single point calibration. All of the compounds had T_{max} values of 90 min. C_{max} and AUC values were rank ordered bilobalide > ginkgolides A > B > C > J. Half-lives ranged from 92 min (bilobalide) to 194 min (ginkgolide C). Using a one-point calibration, similar TK data were obtained except for ginkgolides C and J, which were present at the lowest concentrations. This study indicated that a limited calibration and TK analysis can be utilized to generate useful TK data for a multi-constituent mixture.

2887 Toxicokinetics of Temephos in the Rat: New Evidences of Its Potential Toxicity

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Temephos (Tem) is an organophosphorus larvicide used to control dengue, zika and chikungunya vector. Tem is classified as low toxicity pesticide in acute exposure. Data on the toxicity and disposition of Tem and its metabolites is limited. The aim of this study was to evaluate the disposition and metabolism of Tem in the rat. Adult male Wistar rats were administered with 300 mg/kg Tem emulsified in saline solution by gavage and were sacrificed after treatment. Blood, organs, and tissues were removed for Tem and its metabolites determination after extraction with acetonitrile by HPLC-DAD-MSD. At least twelve metabolites were detected, among them Temephos-sulfoxide (Tem-SO), Temephos-oxon (Tem-oxon), Temephos-oxon-sulfoxide (Tem-oxon-SO), Temephos-dioxon-sulfone (Tem-dox-SO_2), Temephos-sulfone-mono-hydrolyzed (Tem-SO_2-OH), Temephos-oxon-SO-mono-hydrolyzed (Tem-oxon-SO-OH), 4,4´-thiodiphenol (TDP), 4,4´-sulfinyldiphenol (SIDP), and 4,4´-sulfonyldiphenol (SODP) or bisphenol S (BPS). The liver exhibited the presence of greater metabolites number. Tem was the main compound detected in all tissues and was gradually accumulated in fat until 24 h (200.2±27.3 µg/g). Blood concentration data for Tem were fitted to a one-compartment model. After 2 h, Tem blood concentration reached the peak (10.4±1.4 µg/ml, $t_{1/2\ abs}$=0.38 h) and only trace levels were detected at 36 h ($t_{1/2\ elim}$=8.6 h). Tem-SO_2-OH levels remained relatively constant until 36 h and represented the more frequent metabolite observed in blood and kidney. Tem-oxon and -dox were detected in the brain. The clearance of the liver and kidney was 7.59 and 5.52 ml/min. The partition coefficients (organ concentration/blood concentration) of Tem for fat, liver, kidney, and brain were 20.0, 4.6, 2.5, and 1.8, respectively. These results indicate that Tem is biotransformed to reactive metabolites such as mono-oxons, di-oxons, and BPS, a well-known endocrine disruptor. In addition, Tem is well absorbed in rats, is extensively metabolized, widely distributed and preferentially accumulated in fat. These results improve the understanding of Tem toxicokinetics as well as its relationship with the organ-specific toxicity.

2888 Toxicokinetics of Bisphenol-S and Its Glucuronide Conjugate following Oral and Dermal Exposure in Volunteers

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The measurement of bisphenol-S (BPS) and its glucuronide congugate (BPSG) in urine may be used for the biomonitoring of exposure in populations. However, this requires a thorough knowledge of their toxicokinetics. The time courses of BPS and BPSG were assessed in accessible biological matrices of orally and dermally exposed volunteers. Under the approval of the Research Ethics Committee of the University of Montreal, six volunteers were orally exposed to a BPS-d8 deuterated dose of 0.1 mg/kg body weight (bw). One month later, 1 mg/kg bw of BPS-d8 were applied on 40 cm^2 on the forearm and then washed 6 h after application. Blood samples were taken prior to dosing and at fixed time periods over 48 h after treatment and complete urine voids were collected pre-exposure and at established intervals over 72 h post-dosing. Following oral exposure, the plasma-concentration time courses of BPS and BPSG evolved in parallel, and showed a rapid absorption and elimination. Average peak values (± SD) were reached at 0.7 ± 0.1 and 1.1 ± 0.4 h postdosing, respectively. From the elimination phase, average apparent half-lives (t½) of 4.8 ± 0.4 and 4.5 ± 1.1 h were calculated for BPS and BPSG, respectively. Plasma concentration time-courses and urinary excretion rate profiles roughly evolved in parallel for both substances. Average peak values of BPS and BPSG in urine were reached at 2.3 ± 1.0 and 1.7 ± 1.0 h postdosing. Mean (± SD) apparent elimination t½ of BPS and BPSG derived from excretion rate profiles were 5.8 ± 1.2 and 5.0 ± 0.4 h. The average percent (± SD) of the administered dose recovered in urine as BPS and BPSG over the 0-72 h period postdosing were 2 ± 1 and 86 ± 12 %. Following dermal application, plasma levels were under LOQ or LOD at most time points. However, peak values were reached between 5 and 8 h depending on individuals, suggesting a slower absorption rate compared to oral exposure. Similarly, limited amounts of BPS and its conjugate were excreted in urine and peak excretion rates were reached between 5 h to 11 h postdosing. Excretion rate appeared similar between BPS and BPSG. The average percent (± SD) of the administered dose recovered in urine as BPS and BPSG was about 0.005 ± 0.001 and 0.09 ± 0.06 %, respectively. These data provide greater precision on the kinetics of this contaminant in humans and should be useful in developing a toxicokinetic model for a better interpretation of biomonitoring data.

2889 Role of Kinetically Derived Maximum Dose (KMD) in Repeated-Dose Toxicity Studies

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The importance of toxicokinetic (TK) data in understanding systemic exposure is included in OECD Health Testing Guidance and emphasizes that TK data can be used to improve selection of doses for repeated dose mammalian toxicity studies. The Kinetically-Derived Maximum Dose (KMD) approach selects a dose-range more relevant for risk assessment purposes. Doses based on TK data are quantitatively relevant to real-world human exposures as compared to testing at the limit dose. Since use of the KMD approach can result in doses lower than those associated with the long-standing conventional Maximum Tolerated Dose (MTD) dose selection approach, challenges have been raised that this potentially compromises identification of health hazards used in regulatory classification and labeling of chemicals. Chemicals highlighted as case studies are Sulfoxaflor (route selection), 2,4-D (saturated renal clearance and toxicity), Arylex (pharmacodynamic response), ethyl benzene (*post-hoc* study analysis), and ethyl tertiary butyl ether, and afidopyropen (mode of action) where the fold difference between the KMD dose level and human exposure is 40,000; 13,000; 123,000; 5,000; 175,000 and 4,000; respectively. For the case studies, KMD vs MTD doses are compared, values listed after the chemicals represent the KMD selected for a study vs the likely dose via the MTD approach or the KMD from *post-hoc* analysis vs the MTD used in the study: Sulfoxaflor (750, 1000 ppm), 2,4-D (26, 52 mkd), Arylex (157, 244 mkd), ethyl benzene (300, 750 ppm), ethyl tertiary butyl ether (2000, 5000 ppm), and afidopyropen (1000, 3000 ppm). Case studies associated with KMD vs MTD dose selection strategies illustrate the following: 1) Testing at KMD selected dose levels offers appropriate protection of human health (examples include chemicals where KMD dose levels are 3-5 orders of magnitude above human exposure), particularly when knowledge of human exposures is rapidly expanding; 2) KMD is consistent with current knowledge of dose-dependent transitions of toxicity responses; 3) KMD evaluations can be retroactively applied to previous classification/labeling/risk assessments based on data from MTD testing; 4) KMD approach testing honors commitments to reducing animal testing and minimizing animal stress; and 5) The opportunity to remove inter-and intraspecies uncertainty factors exists with knowledge of systemic dose. These examples will illustrate the importance of understanding systemic dose and TK of a chemical and its metabolites in top-dose selection, study interpretation and human relevance.

2890 A Parent-Metabolite Model for Predicting Human Efficacious Dose of (2R,6R)-Hydroxynorketamine, a Metabolite of Ketamine with Antidepressant Effects

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Ketamine has been used as an antidepressant in clinics but its use is limited by its side effects and abuse potential. (2R,6R)-Hydroxynorketamine (2R,6R-HNK) is one of the metabolites of ketamine and has been shown to have rapid antidepressant effects in rodents but without the adverse effects of ketamine. Major metabolites of ketamine have been analyzed in human plasma post ketamine doses; however, 2R,6R-HNK was never administered directly in humans and there is no direct pharmacokinetic (PK) data to estimate the efficacious doses needed in humans. Models reported in literature are not sufficient for prediction of 2R,6R-HNK PK in humans. Preclinical studies have resulted in very different PK properties in rodents and dogs, causing uncertainly for scaling to humans. The current work was to develop a parentmetabolite PK model to describe the PK of ketamine and its metabolites, and estimate the PK parameters of 2R,6R-HNK for prediction of exposure in humans based on the effective dose in mice. The concentration-time profiles and urine excretion data of ketamine and its major metabolites including 2R,6R-HNK were collected from literature by digitalization of the reported plots. The model development was a step-wise approach by building a model for ketamine first and adding metabolites sequentially for estimation of model parameters. Assumptions were taken based on information from literature where data was missing. The developed model was then used for predicting human PK profiles and non-compartmental analysis was used for analysis and comparison of both predicted human PK profiles and mouse PK profiles. The dose in humans was calculated which will provide the same exposure in mice at the effective dose, which was 15 mg/kg as reported in literature. The modeling re-

sults predicted the doses providing equivalent exposure in humans would be 0.05 to 0.2 mg/kg which was lower than the estimated dose using allometric scaling which was 0.4 mg/kg.

2891 Development of a Pharmacokinetic Model for Phosphine Gas Inhalation

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Computational modeling has become increasingly relevant for assessing the kinetics and dynamics of potential drug candidates and toxic industrial chemicals. These models complement *in vitro* and *in vivo* experiments, creating a more comprehensive view of drug efficacy and toxicity while reducing animal use. Physiologically based pharmacokinetic (PBPK) models combine physicochemical properties with physiological data to predict concentration-time profiles within individual tissues. Despite the widespread use of PBPK models, applications involving inhalation of gaseous species remain unexplored. Phosphine gas (PH_3) is a widely used rodenticide and common reagent in chemical synthesis. Despite commercial uses, PH_3 poses a considerable threat to human health. In this study, GastroPlus, a predictive modeling software, was used to develop a PH_3 inhalation PBPK model. Given that the mechanism of action of PH_3 is largely unknown, this model was used to survey the utility of PBPK modeling for refining future studies. A PBPK model was developed to mimic PH_3 inhalation in adult female Sprague-Dawley rats. A concentration-time product of 21450 ppm × min (660 ppm for 32.5 min) was modeled, and delivered doses of PH_3 were calculated using individual subject minute volumes and average measured concentration of PH_3. In order to model exhalation of gaseous species, assumptions regarding the precipitation, dissolution, and clearance of PH_3 in pulmonary compartments were made. Precipitation was assumed to be rapid, dissolution was assumed to be negligible, and metabolic clearance was included to further mimic the exhalatory loss of PH_3. The developed PBPK model was compared to pharmacodynamic (PD) effect-time profiles of observed cardiopulmonary effects, represented as a fraction of baseline values, during and post-exposure. For PD model development, subjects were randomly separated into training and test cohorts. Direct E_{max} models were found to best fit the relationship between plasma concentrations of PH_3 and minute volume (MV) and left ventricular contractility (+dP/dt) with root mean square error (RMSE) of 0.864 and 0.266, respectively. The trained PD models were then applied to the test cohort giving an RMSE of 0.545 for MV and 0.109 for +dP/dt. These relationships imply higher concentrations of PH_3 lead to an increase in both parameters up to a maximum response, which may provide insight into pathways implicated in PH_3 toxicity.

2892 Bioactivation of Halogenated Aromatic Drugs as a Precursor to Drug-Induced Hepatotoxicity

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Halogens are increasingly utilized in drug development, yet their potential impacts on toxic risk through enzymatic bioactivation remain understudied. Chemical reactivity trends indicate halogenated drugs are less likely to undergo bioactivation into reactive metabolites like quinones and thus, pose a decreased toxicity risk. However, the number of hepatotoxic halogenated molecules continues to grow with the introduction of new molecular entities indicating knowledge of halogenated molecule metabolism is insufficient for toxic risk assessment. We hypothesize that the presence, type, and location of aromatic ring halogens impacts the chemical step toward reactive quinone formation (V_{max}), while enzyme affinity (K_m) determines the concentration-dependent reaction conditions. We are testing this hypothesis with a novel integration of bioinformatic, computational, and experimental approaches to assess metabolic bioactivation potential of halogenated molecular scaffolds. First, we extracted 259 halogenated compounds from the FDA DILIrank database. We utilized our Xenosite computational metabolic model to assess quinone bioactivation likelihood. Thirteen compounds were selected for further study due to high model scores and reported DILI. Meclofenamate was the highest predicted ambiguous compound and was supplemented by structurally similar derivatives such as diclofenac, lumiracoxib, and others with varied degrees of DILI risk. We have developed experimental methodologies to adduct quinone metabolites using dansyl glutathione and have established a sensitive method to fluorescently detect these metabolites. Future work will ascertain reaction kinetics and identify responsible enzymes to understand the role of halogens in metabolic clearance and bioactivation contributing to drug-induced liver injury (DILI). Additional work will be conducted using patient health records to identify trends between toxic outcomes and patient populations to stratify patient risk. These findings will provide critical insights on the impact of halogenated compound bioactivation as a precursor to DILI and thus, provide a foundation for better risk assessment in drug discovery and development. *Supported by NIGMS-T32-GM106999/NLM-R01-LM012222.*

2893 Effect of Acute Inhalation Co-exposure of Naphthalene and Carbon Particles on the Systemic Absorption of Naphthalene: Using Excretion of 1-Naphthol as a Biomarker

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Inhalation exposures to semi volatile organic (SVOC) chemicals typically include particulate matter suspended in air. There is almost no information available currently on how particulate matter in air interacts with SVOCs to influence their absorption in the lungs. We found previously that co-exposure of rats to naphthalene and carbon particles increases the naphthalene concentration in lung tissues compared to animals exposed to naphthalene alone. The objective of this study was to examine the effect of carbon particle co-exposure on the absorbed dose of inhaled naphthalene, with urinary excretion of the 1-naphthol metabolite of naphthalene used as a quantitative biomarker of systemic absorption. Male Sprague-Dawley rats were exposed nose-only for 1 h to naphthalene vapor at concentrations of 5, 10, or 20 ppm with or without carbon particles (5mg/m^3). Respiration was monitored during exposure by plethysmography. Following exposure, urine was collected over 24 h intervals for a total of 72 h. Gas chromatography tandem mass spectrometry analysis was used to measure 1-napthol concentration in urine samples. Over 90% of 1-naphthol recovered in urine was collected during the first 24 h. At each naphthalene exposure level, 1-napthol urinary excretion was significantly increased in animals co-exposed to carbon particles. These findings suggest that co-exposure of particles with naphthalene substantially increased its systemic absorption.

2895 The Extent to Which *In Vitro* Distribution Kinetics Determines Differences in Intrinsic Hepatic Clearance between Assay Setups

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In vitro intrinsic clearance assays are used to parameterize physiologically based kinetic models for chemical safety assessment. Usually, for these assays, monolayer cultures of primary human hepatocytes and human hepatic cell lines are used. However, the biotransformation enzymes activity in such cultures is low and/or decreases over time, resulting in underestimation of hepatic intrinsic clearance as compared to *in vivo* situation. For this reason, alternative culture techniques including sandwich cultures and spheroids have been suggested to maintain optimal hepatic functionality. In addition to differences in biotransformation activity, these cultures differ from monolayer cultures in the levels of serum constituents, area and type of exposed plastic and extracellular matrix. Consequently, distribution kinetic processes between these culture models differ and thus hamper the comparison of observed intrinsic clearance across assays. The aim of this study was to quantify and compare the *in vitro* distribution kinetics of intrinsic clearance assays between HepaRG cultured as monolayers, sandwich cultures and spheroids. First, the hepatic function of the culture models was compared using transcriptomics and enzyme activity analysis with probe substrates. Second, the intrinsic clearance of cosmetic ingredients (incl. triclosan, rosiglitazone, butyl paraben) was estimated in the three different cultures. For each culture model, concentrations of chemicals over time were analytically determined in medium, cells, extracellular matrix and plastic of the microplate. Results indicate that spheroid cultures have higher levels of phase I activity, especially CYP3A4 and CYP1A1. Differences in phase II enzymes activity and transporter expression levels between culture models were less pronounced. As a result, intrinsic clearance in spheroid cultures was only higher for chemicals predominantly metabolized by phase I enzymes. Moreover, more lipophilic chemicals and/or chemicals with slower metabolism ($T_{1/2}$ larger than 4h) had lower free concentrations, influencing the measured clearance readout. This is in line with literature and partly explains the underprediction of intrinsic clearance for these classes of compounds.

2896 Variability of Blood/Gas Partition Coefficients Across Species

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Blood/gas partition coefficients of volatile chemical compounds (VCC) are fundamental in inhalation toxicology, anesthesiology, pharmacokinetic modeling, and chemical risk assessment. They are available as empirical but poorly-understood quantities. In particular, their transferability across species remains unknown. In the present work, 528 blood/gas partition coefficients of 101 VCC measured in 7 mammal species were systematically compared using statistical methods. Significant differences across species were identified. On average, the blood/gas partition coefficient was found to decrease with increasing size of the species varying from rat to ox. The dependence was linear on the log scale. The observed correlation does not find a sufficient explanation in terms of water/lipid composition of the blood, but likely, signifies the importance of VCC binding to the peptide/protein component at mass-balance transfer during inhalation. The established relationship can be used for extrapolation of a blood/gas partition coefficient measured in one species to another. This way, multiple independent-study estimates of a human partition coefficient may be obtained and then transformed to a meta-analytical value. A thus-derived partition coefficient represents a meta-analytical estimate reconciled with laboratory measurements in different studies and species, which is a more reliable estimate than of a partition coefficient from a single study. Incorporated in a physiologically-based pharmacokinetic model, a meta-analytical partition coefficient is expected to be more representative of general population, reduce experimental uncertainties and, consequently, increase the accuracy of modeling and improve the effectiveness of public health decisions, for which such modeling is carried out. Thus, this methodology may facilitate public health response, especially under time-limited conditions. *Disclaimer: the findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention/ the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

2897 Influence of Chitosan Coating on the Oral Bioavailability of Gold Nanoparticles in Rats

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Gold nanoparticles (AuNPs) have been extensively studied for a range of applications, including cancer therapeutics. Gold is less toxic than other metallic nanolattices, and gold nanoparticles are excellent conductors of electrical and thermal energy, making it possible to administer non-invasive radiofrequency irradiation with sufficient heat to kill tumor cells. Nanoparticles are generally administered intravenously instead of orally due to negligible oral absorption and cellular uptake. This study evaluated the oral bioavailability of gold nanoparticles coated with chitosan (C-AuNPs), a natural mucoadhesive polymer to eventually investigate the coating agent factor on nanometals bioavailability. The classical method of comparing blood concentrations with area under the curve analysis was used to determine the oral bioavailability of 3 nm C-AuNPs following intravenous and oral doses. The C-AuNPs exhibited an average primary particle size of 3 ± 1.2 nm. Zeta potentials for the C-AuNPs in water were indistinguishable from the neutral measurement: 1.3 ± 0.9 mV. The concentrations of the C-AuNPs administered intravenously (0.8 mg / kg) were declined with half-lives ranging from 15 mins to 24 hours. The serum elimination half-life appeared to be related to the chitosan coating layer, although the half-lives were not significantly different among the treatment groups (p = 40.05). Oral administration of the C-AuNPs resulted in an absorption phase over the first 2 hours after the dose was administered during which blood concentrations increased, followed by an elimination phase. The oral bioavailability for the C-AuNPs was estimated as 2.46 %. In conclusion, even the overall bioavailability of hydrophilic, low molecule cut-off polymer, i.e. chitosan is considerably low, chitosan is a promising candidate to be an oral enhancer for medical nanometallic particles. Further investigation is appreciably needed.

2898 High NRF1 Activity Is Associated with Poor Survival and Resistance to Temozolomide Therapy

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Temozolomide (TMZ) has been widely used as a chemotherapeutic drug for the treatment of new cases of glioblastoma (GBM). However, the development of TMZ drug resistance in almost all cases has become a major clinical problem for the treatment of this lethal disease. The molecular mechanisms of its resistance remain poorly understood. Thus, identification of the cellular and molecular mechanisms that confer drug resistance is an important goal for the treatment of GBM. Here we report the role of nuclear respiratory factor 1 (NRF1) in resistance to TMZ therapy. To our knowledge, there are no studies reporting NRF1 transcription factor activity associated with prognosis of GBM and/or TMZ resistance. Using GBM patient data from the GSE4290 cohort, we observed that patients in the shorter survival duration group had significantly higher NRF1 TF activity (p = 2.36e-31) compared to those in the longer survival duration group. We further confirmed our findings using three independent TCGA, REMBRANDT and GSE16011 GBM cohorts. In each dataset, patients were divided into two groups based on their NRF1 gene expression levels relative to the median NRF1 expression value for that dataset (NRF1 higher than median = high NRF1 group = group 1, and NRF1 lower than median = low NRF1 group = group 2). We confirmed our findings of GSE4290 cohort in these independent studies showing that the high NRF1 group had significantly lower median overall survival (25.9 months, CI = 21 - 33.5) and mean overall survival (49.39 months, CI = 43.3-55.5) compared to the median overall survival (35.9 months, CI = 28.9 - 40.8) and mean overall survival (69.77 months, CI = 60.4 - 79.2) of the low NRF1 group. Kaplan-Meier analysis also showed that high NRF1 gene expression is a risk factor (p = 0.0016) for poor prognosis in glioma. Using TCGA data and focusing on GBM patients treated with Temozolomide, we observed that IDH1 mutation status was a statistically significant determinant of overall survival outcomes, with the longer overall survival group enriched for IDH1 mutations, but none of the other factors such as age, gender, MGMT status, original cellular subtype etc. were significantly different between the two groups by ANOVA analysis. High NRF1 cases of GBM treated with TMZ showed significantly lower survival compared to GBM cases with low NRF1 activity. Further investigation revealed that NRF1 regulated target genes are differentially over-expressed in patients that respond poorly to TMZ based therapy. Differential gene expression analysis was performed on the two groups, and the following 10 NRF1 target genes were found to be upregulated in the high risk/ poor survival outcome group: LDHA, ZMAT3, NSUN2, ARMC5, NDEL1, CLPTM1L, ALKBH5, YIPF5, PPP2CA, and TFG. In summary, our findings suggest an NRF1-based potential mechanism by which GBM patients may acquire resistance to TMZ and targeting NRF1 signature causal pathway may help in overcoming TMZ resistance.

2899 The Use of Normative qEEG Databases Increases the Accuracy of Subject Screening in Phase I Clinical Trials of Drugs with Seizure Liabilities

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Electroencephalography (EEG) is added to drug safety assessments during Phase I clinical trials only when a seizure liability is suspected. In such cases, subject inclusion criteria for healthy volunteers require normal EEG which entails the absence of epileptiform discharges against a normal background EEG activity. This is usually assessed by a visual inspection of short segments of EEG collected under eyes open and closed conditions. While detection of epileptiform discharges can only be conducted visually and has high interrater reliability, this is not the case for subtle nonepileptiform EEG abnormalities, some of which are difficult to interpret even when detected. Based on qEEG (quantitative EEG) advances over the past decades, we can now generate source-correlated 3-dimensonal topographical maps reflecting anatomical integrity of Broadmann areas, modular connectivity, and effective functionality of short and long pathways underlying normal brain function. These were made possible by the development and release into the public domain of LORETA (Low-Resolution Brain Electromagnetic Tomography) EEG source correlation combined with modern tractography, commonly referred to as "brain mapping". These functional qEEG metrics (e.g. spectral, coherence and phase metrics) can be compared with a rigorously developed and validated normative qEEG database (NDB) to ensure that the subjects are within the normal range of EEG variability. The qEEG process entails generating a detailed mapping of all functional areas (cortico-cortical and cortico-thalamic) as well as the connectivity between them, within minutes. The FDA considers the NDB-based qEEG as "useful and beneficial addition to the array of medical tests used to evaluate brain structure and function". Results are statistical deviations (z-scores) displayed in topographical z-score maps. Here we present a stepwise protocol for adding z-scored (deviation from age-matched normative database) qEEG measures, with examples of normal and abnormal nonepileptiform EEG. These added EEG metrics increase the accuracy of EEG screening in Phase I when integrated with clinical exams, and can also serve as a baseline if EEG biomarkers of drug effect are required.

2900 Predicting Human Infection Risk: Do Rodent Host Resistance Models Add Value?

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Use of genetically engineered rodents is often considered a valuable exercise to assess potential safety concerns associated with the inhibition of a target pathway. When there are potential immunomodulatory risks associated with the target, these genetically modified animals are often challenged with various pathogens in an acute setting to determine the risk to humans. However, the applicability of the results from infection models is seldom assessed when significant retrospective human data become available. Thus, the purpose of the current review is to compare the outcomes of infectious pathogen challenge in mice with genetic deficiencies in TNF-a, IL17, IL23, or Janus kinase pathways with infectious outcomes caused by inhibitors of these pathways in humans. In general, mouse infection challenge models had modest utility for hazard identification and were generally only able to predict overall trends in infection risk. These models did not demonstrate significant value in evaluating specific types of pathogens that are either prevalent (ie rhinoviruses) or of significant concern (ie herpes zoster). Similarly, outcomes in mouse models tended to overestimate the severity of infection risk in human patients. Thus, there is an emerging need for more human-relevant models that have better predictive value. Large meta analyses of multiple clinical trials or post-marketing evaluations remains the gold-standard for characterizing the true infection risk to patients.

2901 Carbon Monoxide Poisoning Fatalities from the National Poison Data System, 2013-2017

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In the United States, unintentional exposure to carbon monoxide (CO) results in approximately 50,000 emergency department visits and over 400 fatalities each year (CDC, 2019; Sircar et al., 2015). The purpose of this study was to review the CO exposure and fatality cases reported to US poison control centers (PCCs). Using a retrospective descriptive design, CO exposure data in the 2013-2017 National Poison Data System (NPDS) annual reports were examined for the following variables: age, gender, intent, and the number of yearly fatalities. Analytes and carboxyhemoglobin (COHb) levels of the CO fatality cases were also reviewed when reported. There were a total of 62,205 single CO exposures in the five-year study period, including 283 fatalities (Female 43.1%). No significant annual trends in CO exposure or fatality were identified. The age reported of these fatality cases ranged from 1 to 96 years (M = 42.05, SD = 24.79). Major reasons for fatal CO exposures included unintentional-environmental (73.1%) and intentional-suspected suicide (17.0%). Unintentional environmental CO fatal exposures affected 115 males and 87 females, and the age distribution peaked at the age of 7 (N = 8), 13 (N = 6), and 64 (N = 6). A total of 25 males and 22 females who died of CO exposures were reported as intentional suicide. No children under 16 years were reported in the suicidal intent category and the two peaks in age distribution for this category were 37 (N = 3) and 53 (N =3). Carboxyhemoglobin (COHb) was logically the most frequently used analyte (97.28% of 147 analytes) and the average concentration was 45.16% at the time of autopsy (N = 40). The current study revealed no significant improvement in CO fatal or non-fatal exposures during the five-year study period. Unintentional exposures to CO in the environment accounted for the majority of fatal cases among children as well as adults. Suicides via CO exposure accounted for a noteworthy proportion of fatal cases, mostly in adults. Coupled with information from the CDC CO Poisoning Surveillance Network, these findings have important implications for identifying the scope and potential strategies for CO poison prevention. Suggested directions for continued and innovative awareness campaigns could include public health education with a focus on identified CO sources, safety promotion for National Poison Prevention Week, and partnerships between poison prevention outreach programs, emergency departments, suicide prevention programs, school districts, Women, Infant, and Children (WIC) clinics, and Federally Qualified Health Clinics (FQHC). Such efforts offer the potential for decreasing CO poisoning incidents in at-risk populations.

2903 Methionine Supplemented Diet Worsens Recovery after Stereotactic Body Radiotherapy (SBRT) in a Mouse Model

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Nearly half of all cancer patients undergo radiotherapy (RT) as a treatment modality. However, up to 70% of patients develop radiation-induced gastrointestinal syndrome (RIGS). Symptoms of the latter can be exacerbated by comorbidities, as well as diet and its interaction with the gut microbiome. Methionine (Met) is an essential amino acid required for the majority of key biological processes. At the same time, we and others have shown that Met supplementation may result in a number of toxic effects. This is of particular concern as the typical Western diet contains ~3 times more Met than the daily requirements. Based on our previous studies, we hypothesized that Met supplemented diet (MSD) will exacerbate radiation-induced injury through its effect on the gut microbiome. To test this hypothesis, CBA/CaJ mice were irradiated with the small animal radiation research platform (SARRP) targeting the upper abdomen, thus mimicking SBRT. Mice receiving MSD lost more weight and maintained decreased body weights over the 10-day period compared to mice receiving Met adequate diet (MAD). While no differences were observed histologically, there were significant differences in gut microbiome profiles between the MAD and MSD mice. Furthermore, significant increases in circulating neutrophils with corresponding decreases in lymphocytes were observed in MSD mice at 7 and 10 days post-SBRT, indicating a response to infection. This was confirmed by an increase in bacterial DNA in the liver on day 10. Furthermore, changes in the expression of the tight junction-related proteins *Cldn2*, *Cldn5*, and *Cldn6* indicated a possible increase in intestinal permeability. In conclusion, MSD exacerbates SBRT-induced RIGS by altering the gut ecology and increasing intestinal permeability, thus resulting in an elevated risk of sepsis.

2904 Toxicokinetic Analysis of microRNA-122 in Hepatotoxicity Demonstrates Metabolism to isomiRs and Clearance by Kidney and Spleen

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Circulating microRNAs (miRs) are promising toxicology biomarkers. MicroRNA-122 (miR-122) is an hepatocyte-specific biomarker of drug-induced liver injury (DILI). Our first objective was to determine the tissue distribution of miR-122 after its release from the liver using a mouse model of acetaminophen (APAP)-DILI. RNA sequencing has revealed that miRs can exist in multiple isoforms ('isomiRs') that vary in length. Our second objective was to profile miR-122 isomiRs and to adapt a novel assay (Dumbbell-PCR; Db-PCR) to selectively quantify isomiRs. Male C57BL/6J mice were treated intraperitoneally with APAP (300mg/kg) or vehicle. miR-122 was measured in blood plasma and organs using PCR. The presence of miR-122 isomiRs was determined by small RNA sequencing. Synthetic canonical miR-122 (22 nucleotides) and its isomiRs underwent PCR using SYBR green and TaqMan protocols. The Db-PCR assay was optimised for the detection of miR-122 isomiRs. At 6h after APAP treatment liver miR-122 expression decreased and circulating miR-122 increased ($1.6x10^5$ and $3.2 x10^8$ median miR-122 copies/µL plasma for control and treatment groups, respectively; $P < 0.0001$). At 12h after treatment circulating miR-122 had returned to control levels. With APAP-DILI, miR-122 expression increased in the renal cortex ($2^{-dCt} = 0.04$ and 0.1 for vehicle and treatment groups, respectively; $P < 0.05$), renal medulla ($2^{-dCt} = 0.07$ and 0.1 for vehicle and treatment groups, respectively; $P < 0.05$), and the spleen ($2^{-dCt} = 0.005$ and 0.03 for vehicle and treatment groups, respectively; $P < 0.001$). FACS analysis demonstrated that miR-122 increased specifically in renal tubular cells. Small RNA sequencing demonstrated the presence of multiple miR-122 isomiRs, with 3' variants being in high concentration. The efficiency of SYBR and TaqMan PCR decreased substantially as 3' nucleotides were removed. By contrast, Db-PCR was able to discriminate equimolar isomiRs from canonical miR-122 with a difference of around 10 cycle threshold (C_T) values. In summary, DILI induces the transfer of multiple isomiRs of miR-122 from liver to the kidney and spleen that 'industry standard' PCR kits cannot accurately quantify. Db-PCR is a novel PCR-based assay that should be taken forward to full validation.

2905 Acute Toxicity Evaluation of a Novel Ceramide Analog

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The present study aims to determine the *in vivo* acute toxicological profile of the synthesized ceramide analog 315, (S,E)-3-hydroxy-2-(2-hydroxybenzylidene)amino-N-tetradecylpropanamide. Analog 315 is a novel ceramide which was synthesized in our laboratory for the potential treatment of breast cancer. Treatment of nude mice with 10mg/kg of the Analog, 5 days per week for 3 weeks, was shown to inhibit tumor growth, and also to reduce metastasis of triple negative breast tumors caused by MDA-MB-231cells. Hence, a subsequent study was performed to determine the toxicological profile of this compound in nude mice. In our first toxicity study, nude mice were treated with 0, 40, 80 or 120 mg/kg body weight given only once. During this 14-day acute toxicity testing period, there was no mortality or morbidity except that the calcium concentration in the serum was significantly higher in the high dose group. Therefore a second acute toxicity study was done with lower drug doses, in which mice were treated with a single dose of 0, 25, 50 or 75 mg/kg body weight. During this 14-day observation period, conventional toxicity evaluation showed no abnormal signs of toxicity (No changes in body weights, organ weights, or behavior), and all biochemical parameters evaluated including serum calcium concentration were within the normal range. Our results are expected to pave the way for developing novel ceramide drugs with more efficacy and less toxicity than conventional chemotherapies for treating breast cancer, and improve the quality of life in patients.

2906 *In Vitro* EGFR-TKI Acquired Resistance in Brain-Seeking PC-9 Cells Overcome by a Bcl-2 Inhibitor

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A lung cancer PC-9 brain seeking cell line (PC-9-Br) was established by a repeated *ex vivo* brain extraction method. PC-9-Br showed a significant resistance to epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) gefitinib compared with parental PC-9 by *in vitro* MTT assay. Further studies on the molecular mechanisms of gefitinib resistance revealed that EGFR and phospho-EGFR (p-EGFR) significantly decreased in PC-9-Br, while Bcl-2 significantly increased in PC-9-Br compared with parental PC9. However, the expressions of E-cadherin and vimentin did not show epithelial-mesenchymal transition (EMT) in PC-9-Br compared with parental PC-9. Additionally, gene analyses showed that PC-9-Br had neither T790 mutation (T790M) nor amplifications of MET and HER2 compared with parental PC-9. Therefore, our study demonstrated that brain metastases of lung cancer cells may independently prompt drug resistance without any drug treatment. Moreover, we found that the combination of a Bcl-2 inhibitor ABT263 at low and non-toxic concentrations with gefitinib showed significant synergistic effects on overcoming the acquired resistance of PC-9-Br *in vitro*. The results suggest that Bcl-2 inhibitors may have a great potential in clinical therapeutic applications for treatments of lung cancer brain metastasis and relevant acquired resistance.

2907 Cosmetics Europe Eye Program: Application of Two Defined Approaches for Ocular Toxicity Predictions Based on *In Vitro* Bottom-Up Approach on 4 Case Studies

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The eye irritation/severe eye damage historically played a key role in the development, validation and regulatory acceptance of *in vitro* test methods and remains an active topic for regulatory toxicology, currently within the frame of the Organisation for Economic Cooperation and Development (OECD), Test Guideline Program. Recent activities in developing defined approaches (DAs) and how those fit have been achieved for liquids chemicals (Alépée et al., 2019 a,b). From the scientific side, the DAs allow to perform predictions over the entire spectrum of United Nations Global Harmonized System for classification and labelling (UN GHS Categories 1, 2 and No Category), by strategically combining physico-chemical properties to sequential *in vitro* testing on Reconstructed human Cornea-like Epithelium (RhCE) and Bovine Corneal Opacity and Permeability Laser Light-Based Opacitometer (BCOP LLBO) in a first DA bottom-up approach; and BCOP LLBO and short time exposure (STE) test in a second DA bottom-up approach. In the present work, application of both DAs is exemplified with specific case studies on four chemicals. Among the tested chemicals, a chemical (1,3-di-isopropyl benzene) known to be No Cat. *In vivo*, is predicted as No Cat. in the first tier strategy test methods of the DAs. Concerning the positive calls on the 2 known *in vivo* Cat. 2 chemicals (2-Ethyl-1-Hexanol and 2-Methyl-1-Pentanol) and an *in vivo* Cat. 1 chemical (2-Hydroxy-Isobutyric acid ethyl ester), the second-tier step which consists of their evaluation in the BCOP LLBO test which sub categorized them as Cat 2 / Cat. 1, respectively. In conclusion, these case studies reflect 2 approaches on how to move from animal testing into an evaluation of new ingredients based on examples of application of DAs on an Integrated Approach for Testing and Assessment for safety purposes of ingredients.

2908 Testing for Cosmetics Safety Assessment: Evaluation of Results from over 70 *In Vitro* Eye Irritation Studies

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There are typically two main steps involved in cosmetics safety assessment: 1) theoretical evaluation of formulation ingredients and raw materials to determine local and systemic toxicity potential, and 2) testing to confirm local toxicity effects of final formulations. Interactions among ingredients are not always easily predicted by single-ingredient theoretical evaluations – this is where testing for safety is critical. Given the momentum regarding refinement, reduction, or replacement (3Rs) of animal-based tests with *in vitro* methods, it is important to define testing strategies that meet those requirements and provide confident safety assurance for each toxicity endpoint (e.g., eye irritation potential). When used in combination, the Chorioallantoic Membrane Vascular Assay (CAMVA) and Bovine Corneal Opacity and Permeability (BCOP) assay have demonstrated to be relevant and reliable methods to predict eye irritation potential of cosmetics. These assays aid in predicting the possible effects of mixtures in the human eye because, together, they represent the majority of the eye (i.e., conjunctiva and cornea). In the BCOP test, there are currently two suggested classifications at the low end of the irritation spectrum: 1) GHS (no category) and 2) Gautheron (mild irritant). Results from our 70 test batteries have been used to establish a prediction model for safety assessment, which can be used to guide decision-making regarding warning labels. According to our data analyses, a formulation could be confidently predicted as "practically non-irritating" to the human eye when the CAMVA RC_{50} is >70% and BCOP *in vitro* Irritancy Score (IVIS) is ≤3. Comparing *in vitro* test results predictions with post-marketing surveillance analyses allows evaluation of the accuracy of the two-test battery prediction model, and the effectiveness of safety evaluation recommendations for product market release, all to better ensure consumer safety.

2909 Toxic Effect of E-cigarette on Blood Retinal Barrier (BRB) in Diabetic Macular Edema Model

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Growing popularity of Electronic cigarette (E-Cig) as a safe alternative to traditional tobacco smoking has prompted the regulatory authorities and researchers towards studying the effect of E-Cig on health. Several groups including our lab have shown that E-Cig contains different potentially toxic compounds along with nicotine which may promote cerebrovascular and cardiovascular diseases. In this respect, diabetic macular edema (DME), is characterized by macular area thickening and breakdown of blood retinal barrier (BRB), plays a pivotal role in losing vision in patients affected by diabetic retinopathy. Hyper glycemia and hypoxia are the key elements behind the progression of this disease. Previous studies found that nicotine promotes blood retinal barrier (BRB) damage in an *in vitro* BRB model however, the overall impact of E-Cig vapor, on BRB integrity and function has not been investigated yet. Therefore, the aim of this study is to evaluate the effects of E-Cig on BRB by determining its impact on BRB integrity/permeability, and HIF/VEGF expression in retinal pigmented epithelial cells (ARPE-19) as a DME model. For this study, ARPE-19 cells were grown on semipermeable Transwell supports. TEER FITC-dextran permeability assays were used to assess BRB integrity. To mimic the E-cig exposure, we used freshly prepared E-Cig extracts at physiologically relevant concentrations according to a previous validated published protocol. Our preliminary results strongly suggest that E-Cig extracts alters outer BRB integrity in a hyperglycemic and hypoxic microenvironment mimicking diabetic conditions. Loss of BRB integrity was paralleled by increased

VGEF expression. From this preliminary data, we can infer that, chronic E-Cig vapers may be at risk of onset/progression of DME. Additional studies are ongoing to determine the underlying mechanisms through which E-Cig impairs BRB.

PS 2910 Ocular Side Effects of Systemic Medications: Utilization of the 3D Human Corneal Epithelial Tissue Model

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Many systemic medications at high doses have a potential to induce unwanted ocular effects, including light sensitivity, pain, or corneal edema that are due to inflammation and/or cytotoxicity. Current studies utilize animal models which are not suitable for rapid drug screening, have poor species extrapolation and standardization. There is a need for physiologically relevant, human primary corneal epithelial tissue models to address ocular safety for the evaluation of new drug formulations. We have utilized the 3D human corneal epithelial tissue model, EpiCorneal, to analyze the effect of common drugs with known adverse ocular side effects. EpiCorneal tissues are comprised of normal human corneal epithelial cells, express site-specific mucins and tight junctions and attain morphology, barrier properties (TEER>900±200 Ω*cm^2), and gene expression similar to the *in vivo* human cornea. The effect of physiologically relevant concentrations of Chlorpromazine hydrochloride (CPZ), a common pshychtropic agent; Hydroxychloroquine sulfate (HCQ), an anti-inflammatory and anti-malaria drug (HCQ); and Fosamax (Alendronate Sodium, FOS), a common anti-osteoporosis agent, were investigated. Effects on tissue viability (MTT assay), barrier function (Transepithelial electrical resistance, TEER), histology, LDH and cytokine release were studied. EpiCorneal tissues were incubated in the medium containing CPZ at 6.25 - 100 µM, HCQ at 6.17 - 500 µg/ml, or FOS at 0.1 - 100 µg/ml for 24h and 48h. For CPZ-treated tissues the lowest dose to cause a significant decline in barrier function (67.4%) was 12.5 µM at 24h, and 25 µM decreased tissue viability (60.5%) at 48h. For HCQ-treated tissues, a decline in TEER (67.4%) was detected for 18.52 µg/ml at 24h, and in tissue viability (53.6%) for 55.56 µg/ml at 48h. For FOS-treated tissues, a significant TEER decrease (57.8%) was detected at 0.1 µg/ml and in tissue viability (85.7%) for 10 µg/ml, both at 48h. Treatment-specific changes in tissue morphology and dose response of LDH and cytokine release were also observed. EpiCorneal tissue model has been very useful for evaluating formulations with negligible irritation potential. It will model systemic drug exposure for extended time, will generate rapid response, avoid species extrapolation, be more cost effective and more reproducible than animal methods, and will facilitate drug discovery by allowing screening and optimization of pharmaceuticals prior to clinical studies.

PS 2911 Chronic O-GlcNAcase Inhibition Causes Lens Opacities in Dogs but Not Rats

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Cataracts are the leading cause of blindness worldwide and diminished proteolytic functionality in the lens may cause cataracts. O-linked β-N-acetylglucosamine (O-GlcNAc) has been proposed as an endogenous inhibitor of the proteasome and transgenic mice that over express a dominant negative form of O-GlcNAcase, GK-NCOAT, in the lens, had suppressed removal of O-GlcNAc from lens proteins, resulting in increased O-GlcNAc and decreased proteasome function in the lens, markedly larger nuclear cataracts than control mice, lens epithelial cell hyperplasia, and inhibition of lens fiber cell denucleation. These results suggested that increased O-GlcNAc in the lens could lead to cataract formation. As a potential treatment for Progressive Supranuclear Palsy (PSP), Merck investigated the potential toxicity of MK-8719, an O-GlcNAcase (OGA) inhibitor in repeat-dose general toxicology studies *in vivo* in Crl: WI(HAN) rats (up to 6 months) and Beagle dogs (up to 9 months). In dogs but not rats, upon ophthalmoscopic examination, MK-8719 produced lens changes, consisting of prominent posterior subcapsular suture lines and/or lens opacities (posterior subcapsular or subcapsular/capsular). These changes were identified in dogs following 4-6 months of dosing with MK-8719 and correlated with histomorphological findings of posterior subcapsular central lens fiber degeneration in some of the animals with ophthalmoscopic findings. To evaluate if the lens changes were mechanism based, ophthalmoscopic examinations were performed on beagle dogs receiving a structurally unrelated OGA inhibitor. This compound produced similar posterior lens changes in dogs following a similar duration of dosing, supporting the conclusion that the lens effects are mechanism based. These findings provide further evidence that increased O-GlcNAc protein posttranslational modifications in the lens can cause cataracts *in vivo* and may have implications for cataract formation as a complication of diabetes since O-GlcNAc is derived from glucose.

PS 2912 Longitudinal Imaging Reveals Patterns of Neurodegenerative Inflammation in Rotenone-Induced Retinal Neurotoxicity

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Rotenone is a mitochondrial toxicant known to induce neurodegeneration. However, the neuroinflammatory responses following exposure to rotenone have not yet been clearly characterized. As resident immune cells of the central nervous system, microglia are known to enhance the neurotoxicity of rotenone. We postulated that non-invasive imaging of the retina can reveal neurotoxic effects of localized exposure to rotenone, including the temporal changes due to neuroinflammation. To test this, we monitored the spatial and temporal changes in retina inflammation and neurotoxicity following direct exposure to rotenone by intravitreal injection, by combining imaging techniques including optical coherence tomography (OCT) and confocal scanning-laser funduscopic (cSLO). To visualize microglia activation, we used Cx3Cr1^{eGFP} transgenic mice in which macrophages express green fluorescent protein. Accordingly, we utilized a non-invasive imaging protocol to assess neurotoxicity longitudinally, without an invasive biopsy. The OCT images were processed to characterize and quantify rotenone-induced changes in retinal morphology. We found that a 0.3µL/10mM concentration of rotenone caused retinal thinning and neurodegeneration, during the late phase of inflammation. Retinal thinning at day 7 correlated to the first appearance of hyper-florescent puncta in the photoreceptor layer. Furthermore, the puncta proliferated at day 14, indicating lipid peroxidation. The Cx3Cr1 microglia, at day 7 post treatment, were aligned with the nerve fiber tracts. Before 7 days, microglia activation in the inner portion of retina was not detected. Based on the OCT findings, neurodegeneration was 7-14 days post-injury, and photoreceptor loss was not evident until day 14. These studies showed a predominantly M1 verses M2 microglia phenotype at 7 days, suggesting transition into chronic inflammation rather than resolving phase. These studies indicated that a low dose of rotenone can facilitate development of a neurodegenerative pathology over time, and that the late phase M1 microglia activation may be the driving force of the delayed response and subsequent neurodegeneration. Furthermore, we believe that the non-invasive imaging used in the current study provides a novel approach for characterizing the inflammatory progression of neurotoxicity. *NIEHS T32ES007254.*

PS 2913 Optical Coherence Tomography (OCT) *In Vivo* Imaging Tool to Monitor Retina in Preclinical Species

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Optical Coherence Tomography (OCT) is a non-invasive high-resolution, 3D-imaging technique with important biomedical application in ophthalmology. OCT provides high resolution (~ 3 microns) volumetric retinal images suitable for morphologic assessment and longitudinal in-life monitoring. OCT has become a valuable tool for the study and diagnosis of retinal diseases in humans, and it may be utilized to evaluate anterior segment structures as well. In drug development, OCT can be utilized in preclinical species to provide a monitoring capability for humans. In this presentation, we report on the utility of spectral domain OCT (Envisu™ R-2200 Model, Bioptigen Inc./Leica Microsystems) to detect changes in eye structures in living rats and mice. OCT was successfully used to measure retinal thickness in-life in a light-induced retinal atrophy model and in a drug-induced (Abilify/aripiprazole) retinal atrophy model in rats. Additionally, using ritonavir-treated mice, OCT was also used to measure retinal pigmented epithelium (RPE) thickness over time. OCT was able to detect treatment-related decreases in retinal thickness and increases in the RPE thickness enabling in-life monitoring and the ability to detect the progression of lesions in the same animal, thereby enabling the use of fewer animals in some preclinical studies and demonstrating a monitoring capability for clinical studies.

2914 The Role of CYP2B6 in Sertraline-Induced Toxicity in Hepatic Cells

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Sertraline is an antidepressant commonly used to manage mental health symptoms relating to depression, anxiety disorders, and obsessive-compulsive disorder. The use of sertraline has been associated with rare but severe hepatotoxicity. Previous research has demonstrated that ER stress, mitochondria impairment, and apoptosis are involved in sertraline-associated cytotoxicity. It has also been demonstrated that sertraline is mainly metabolized by cytochrome P450 (CYP) 2B6. However, the role of CYP-mediated metabolism in sertraline-induced toxicity remains unclear. In this *in vitro* study, we used our established CYP2B6-overexpressed HepG2 cell line to investigate the role of CYP2B6 in sertraline-induced cytotoxicity. We found that the metabolism catalyzed by CYP2B6 significantly attenuated sertraline-induced cytotoxicity in comparison with vector control cells, as measured by LDH and MTS assays. Western Blot analysis demonstrated that less induction of γH2A.X, a hallmark of DNA damage, occurred in CYP2B6-overexpressed cells in comparison to the vector control under the same treatment of sertraline. These findings indicate that CYP2B6 plays a role in the metabolic detoxification of sertraline by preventing DNA damage. This work contributes to our overall understanding of the role that metabolic enzymes play in sertraline-associated hepatotoxicity.

2915 Gut Microbiome Critically Impacts PCBs-Induced Changes in Hepatic Transcriptome Metabolic Fingerprints

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Polychlorinated biphenyls (PCBs) are ubiquitously detected in the body and have been linked to numerous diseases. The liver serves as a central hub for the metabolism of xenobiotics and metabolites. Xenobiotics induced gut dysbiosis is recognized as a critical regulator of disease susceptibility. Although the acute toxicity of PCBs on the liver is known, the role of the microbiome in PCBs-mediated responses remains understudied. We hypothesized that PCBs-mediated changes in the metabolic fingerprints and hepatic transcriptome are regulated by enterotype and dose. Ninety-day-old female conventional (CV) and germ free (GF) C57BL/6 mice were orally exposed to the PCB Fox River Mixture (synthetic PCB mixture, 6 or 30 mg/kg) or corn oil (vehicle, 10 ml/kg), once daily for 3 consecutive days. Organs were collected 24 hours after the final dose. RNA-Seq was conducted in liver, and endogenous metabolites were quantified in liver and serum by LC-MS. Enterotype was the primary factor in clustering the transcriptomic and metabolomic signatures within the same exposure. The numbers of PCB-regulated genes were higher in CV than in GF conditions. The mRNAs and proteins of the prototypical target genes of the major xenobiotic sensing transcription factors AhR, PXR, and CAR were more readily induced by PCBs in CV than in GF conditions, indicating the effect of PCBs on the hepatic transcriptome act partly through the gut microbiome. In addition to the dysregulation of genes important for xenobiotic biotransformation and inflammation, the folding of incorrect proteins pathway was down regulated by PCBs, especially in CV conditions. At the high PCB dose, NADP and arginine, which are known to involved in the mechanism reactions of the drug-metabolizing enzymes (Cyp1-3 family, DhcR7, and Nqo1), were down-regulated in CV mice. In PCB-exposed GF groups, hepatic glucose was up-regulated, whereas fructose 6-phosphate and glucose 6-phosphate were down-regulated, indicating increased glucose utilization potentiated by lack of gut microbiota. In conclusion, our findings demonstrate that habitation of the gut microbiota drives PCBs-mediated hepatic responses, possibly due to crosstalk between gut and liver through the enterohepatic circulation.

2916 Metabolism of Naphthalene by Mouse Liver Microsomes: Glutathione Does Not Affect 1,2-Dihydrodiol Formation

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Naphthalene (NA) is a ubiquitous carcinogenic pollutant to which humans are widely exposed. A prerequisite for NA's toxicity in the respiratory tract is bioactivation by cytochrome P450 (CYP) enzymes. NA metabolic pathways to toxicity involve initial formation of NA-oxide, and subsequent formation of reactive metabolites derived from 1,2-NA-dihydrodiol (producing 1,2-naphthoquinone) or 1-naphthol (producing 1,4-naphthoquinone). However, the relative importance of these reactive metabolites and more specifically the role of reduced glutathione (GSH), which reacts with NA-oxide to form NA-GSH adducts, in modulating the abundance of 1,2-NA-dihydrodiol and 1-naphthol is unclear. In this study, we examined the effects of GSH and UDPGA (cofactor for UDP glucuronyl transferase) addition on the rates of 1,2-NA-dihydrodiol and 1-naphthol formation in reactions of NA with mouse liver microsomes. Levels of 1,2-NA-dihydrodiol and 1-naphthol were measured using UPLC-UV, whereas those of NA-GSH were measured using LC-MS/MS. We show that addition of GSH up to 10 mM, which led to maximal formation of NA-GSH, did not significantly decrease rates of 1-naphthol or 1,2-NA-dihydrodiol formation *in vitro*. On the contrary, UDPGA addition resulted in significant decreases in 1-naphthol formation, but not 1,2-NA-dihydrodiol formation. These results suggest that GSH cannot prevent formation of 1,2-NA-dihydrodiol, a reaction catalyzed by microsomal epoxide hydrolase. The implication from these findings is that levels of NA-GSH may not correlate with those of 1-naphthol or 1,2-NA-dihydrodiol *in vivo* in NA-exposed individuals, and that the risks of toxicity from the three different bioactivation pathways (via the formation of NA-oxide, 1,2-NA-quinione, and 1,4-NA-quinone) should be assessed independently. *Supported in part by NIH grant ES020867.*

2917 Crystal Structures of 2-Nitro- and 3-Nitro-4-Acetamidophenols: Significance to Mechanisms of Pharamacology and Toxicology of 4-Acetamidophenol

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We have previously shown that the putative free radical products of the peroxynitrite anion-CO2 reaction (•NO2 and CO3•⁻) can constitute an important source of non-CYP450-mediated oxidative biotransformation of 4-acetamidophenol (4-AP) and other xenobiotics (clozapine). In reactions of 4-AP/PN/CO2, we find that 2-nitro-4-acetamidophenol (2-nitro-4-AP) is one of the major products formed along with N,N′-(6,6′-Dihydroxy[1,1′-biphenyl]-3,3′-diyl) bisacetamide (dimer of 4-AP) and a metastable N-acetyl-1,4-benzoquinone (NBQI; demonstrated through its binding to N-acetyl-L-cysteine). It was shown recently that NBQI can react with electrophiles like the nitrite ion and form an yet another nitroproduct of 4-AP, viz., 3-nitro-4-acetamidophenol (3-nitro-4-AP). Although we did not find evidence for the formation of 3-nitro-4-AP in 4-AP/PN/CO2 reactions, we believe that 3-nitro-4-AP along with other oxidation products of 4-AP may play a role in the pharmacology and toxicology of 4-AP (4-AP overdose, either accidental or inadvertent, is the most common cause of hepatic failure in the US and elsewhere). Towards understanding this, we have synthesized the 2-nitro and 3-nitro isomers of 4-AP and examined their single crystal properties. To our surprise, we find that the two isomers have quite different molecular planarities in the solid state and also differ in their hydrogen bonding patterns. In 2-nitro-4-AP, the acetamido group is twisted out of the plane of the phenyl group by 9.0° and the nitro group is twisted out of the phenyl plane by 11.8°. The N-H group forms an intermolecular hydrogen bond to acetamido O having N···O distance 2.9079(17) Å and N-H···O angle 176.6(19)°. The OH group forms a bifurcated hydrogen bond, with intramolecular component to the adjacent nitro group (O···O 2.6093(17) Å) and a longer intermolecular component to nitro oxygen (O···O 3.1421(17) Å). In 3-nitro-4-AP, the molecule is nearly planar, with all nonhydrogen atoms having a mean deviation from coplanarity of 0.035 Å. The acetamido group has the largest deviation, with a 5.1° twist about its central N-C bond. The N-H group forms an intramolecular hydrogen bond to nitro O having N···O distance 2.6363(15) Å and N-H···O angle 139.6(15)°. The hydroxy group makes an intermolecular hydrogen bond to acetamido O having O···O distance 2.7183(14) Å and O-H···O angle 172.0(18)°, forming chains. Combined with the recent revelations of mechanisms of action of 4-AP through indirect activation of CB1 receptors by 4-aminophenol (hydrolysis product of 4-AP) and endocannabinoid reuptake inhibitor AM 404, the information presented here may provide useful insights into molecular targets for 4-AP and its metabolites.

2918 Enzyme Kinetic Parameters for Hydrogen Peroxide Generation (Auto-Oxidation) in P450-Related Microsomal Electron Transport Chains

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It is well recognized that a microsomal electron transport chain with terminal oxidase cytochrome P450 enzymes (CYP's) generates hydrogen peroxide. This reaction requires NADPH and oxygen and proceeds via autooxidation without metabolizing substrates or by uncoupling with metabolizing substrates. Rates of hydrogen peroxide generated by this system in rat liver microsomes depend on multiple factors including the specific set of expressed CYP's. Earlier studies by our laboratory showed that the CYP3A family is one of the most active CYP's producing hydrogen peroxide. Of the CYP3A family, rat liver microsomes from male Sprague Dawley (SD) rats contain only CYP3A2; using the Amplex Red/HRP assay, we found that the Vmax for hydrogen peroxide generation by these microsomes in the absence of substrates was 4.0 nmol/min/mg protein with NADPH as the electron donor. Significantly increased hydrogen peroxide generating activity was found in liver microsomes from dexamethasone treated male rats where CYP3A1 and CYP3A2 are highly inducible (Vmax = 14-16 nmol/min/mg protein). Clotrimazole, a form-selective inhibitor of rat CYP3A1 and CYP3A2, suppressed 60% of hydrogen peroxide generation in liver microsomes from dexamethasone treated male rats. The Km for NADPH for hydrogen peroxide generation was similar (≈ 2.0 μM) in microsomes from control and dexamethasone treated male rats. These data indicate that increases in content of CYP 3A family enzyme in rat liver microsomes are largely responsible for the higher rates of hydrogen peroxide formation. Moreover, similarities in the Km for NADPH may represent similar affinities of CYP3A subfamily enzymes for the NADPH-cytochrome P450-reductase during the formation of hydrogen peroxide. *Supported by NIH grants U54AR00573 and R25ES020721.*

2919 Identifying Sites of Interdomain Interaction on Dimeric BM3 P450 Using Covalent Cross-Linking and Mass Spectrometry

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CYP102A1 (BM3) is a model P450 used to elucidate the mechanisms of enzymatic turnover in fusion P450 enzymes. BM3 is active as a homodimer, with dimerization interfaces contained within its N-terminal oxygenase and C-terminal reductase domains. While crystal structures have been successfully resolved for the heme-containing oxygenase domains in complex with a single subunit of reductase domain, the nature of interactions between full-length BM3 subunits in solution are not understood. In this study, we utilized a novel method to identify interactions between BM3 subunits by covalent crosslinking and mass spectrometry (CXL-MS). Specifically, we exposed full-length dimeric BM3 to an MS-cleavable crosslinker disuccinimidyl dibutyric urea then excised and analyzed both monomeric and dimeric BM3 from a single crosslinked sample, then validated assigned crosslinks using recent high-resolution cryogenic electron microscopy (Cryo-EM) structures of the full-length BM3 from our lab. All 13 unique crosslinks present in the monomeric BM3 band were also detected in the dimeric band and mapped to distances on the Cryo-EM structure within the spatial restraints of the crosslinker. The dimeric band yielded 32 unique crosslinking sites, 5 of which were interdomain. The interdomain crosslinks suggested extensive interaction between oxygenase and opposing reductase domains in the homodimer, which is consistent with the understood mechanism of electron transfer in the holoenzyme. These data enhance our understanding of the BM3 homodimer's structural arrangement during enzymatic turnover in solution, and identify novel target sites for enzymatic regulation. *This work was supported by NIEHS training grant T32-ES007062, NIH grants GM077430 and GM007767, NINDS grant NS055746, and the University of Michigan Medical School's Protein Folding Disease Initiative.*

2920 Single Crystal Structure of 2,5-Dinitro-4-Acetamidophenetole: Implication to Phenacetin Toxicity

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The analgesic use of phenacetin (4-acetamidophenetole; 4-AcP) predates the First World War. 4-AcP was probably the first synthetic chemical to go on the market as a fever reducer as well. However, due to its carcinogenic and kidney-damaging properties, 4-AcP was withdrawn from the markets word over some three decades ago. In view of 4-AcP's similarity to cocaine in physical appearance and texture, in recent years, there have been several instances of 4-AcP being used as an adulterant or cutting agent, meaning that 4-AcP is still in use disguised in the form of an illicit drug. Ironically, 4-acetamidophenol, a metabolite of 4-AcP has similar analgesic properties but without any carcinogenicity or kidney toxicity. Although there has been some research on the phase I and phase II metabolites of 4-AcP, the information on metabolites of 4-AcP that could be formed in reactions with cellular oxidants such as hypochlorite ($^{-}$OCl)/hypochlorous acid (HOCl; pKa≈7.53) and the peroxynitrite ($ONOO^{-}$)/peroxynitrous acid (ONOOH; pKa≈6.2; ONOOH and $ONOO^{-}$ are collectively referred to as peroxynitrite or PN) is scanty. We have shown, for instance, that 4-AP forms nitrated and chlorinated metabolites in these systems under physiologically relevant conditions of pH and CO2 concentration (important in the case of reactions with PN). We suspect that a similar set of metabolites may be formed in reactions of 4-AcP with HOCl/OCl and PN±CO2. Towards understanding this and to shed light on molecular targets, we have synthesized 2,5-dinitro-4-acetamidophenetole (2,5-dinitro-4-AcP) using nitric acid-sulfuric acid mixtures in the cold. The single crystals of 2,5-dinitro-4-AcP from methanol were analyzed using the technique of X-ray diffraction. We find that the ethoxy group is nearly coplanar with the phenyl ring, having C-C-O-C torsion angle 1.4° and C-O-C-C(Me) torsion angle 174.6°. The acetamido group is also nearly coplanar with the phenyl ring, having C-C-N-C torsion angle 3.2°. The nitro group adjacent to acetamido is twisted out of plane by 24.2°, and the one adjacent to ethoxy is twisted out of plane by 43.0°. The N-H group forms a bifurcated hydrogen bond, with intramolecular component to the adjacent nitro group (N···O 2.6857(6) Å) and a longer intermolecular component to the other nitro group (N···O 3.4308(6) Å), forming chains. The results of the present study, together with the recent understanding of the mechanisms of action of 4-AP which proceeds through hydrolysis and subsequent formation of arachidonic acid conjugates and their binding cannabinoid receptors, may be useful in providing insights into molecular targets for 4-AcP and its metabolites.

2921 Reactions of Penicillins G and V with Peroxynitrite (±CO_2): Implications to Tyrosine Nitration in Nitric Oxide-Producing Biological Systems

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Peroxynitrite (PN) is an elusive oxidant that can be formed in nitric oxide ($^{\cdot}NO$) producing biological systems from the reaction of $^{\cdot}NO$ with superoxide ($O_2^{\cdot-}$) and oxyhemoglobin. In biological milieu, only a few substrates such as heme proteins and peroxidases react rapidly enough with PN, before it is trapped by the reaction with CO_2 forming $^{\cdot}NO_2$ and $CO_3^{\cdot-}$ free radicals in yields up to 40 mol% of the original PN. Both $^{\cdot}NO_2$ and $CO_3^{\cdot-}$ are highly reactive towards a variety of biomolecules including lipids, proteins, nucleic acids, and antioxidants. For years, our primary goal has been to elucidate the mechanisms of non-P450-mediated oxidative biotransformation of xenobiotics by $^{\cdot}NO$-derived oxidants. With this in view and also in view of the recent demonstration that certain antibiotics interference of protein nitration, we undertook studies of penicillin G (PG) and penicillin V (PV) reaction with PN, PN±CO_2, and PN±CO_2±lysozyme (lysozyme was model protein used in these studies). At pH 7.2 and 37±0.5 ^{0}C, the second-order rate constants for the PN-PG and the PN-PV reactions were found to be 101 and 135 M^{-1} s^{-1}, respectively. These rates are 3- to 4-orders of magnitude smaller than those typically found in reactions of PN with CO_2, hemoproteins, and peroxidases. Also, since the therapeutic concentrations of PG and PV are generally in the μM to sub-millmolar range, we believe that these antibiotics do not react directly with PN in competition with any of three classes of biological target molecules referenced above. To test if the products of PN±CO_2 ($^{\cdot}NO_2$ and $CO_3^{\cdot-}$) react with PG and PV, we performed the direct reactions PG and PV with PN±CO_2 and examined for the formation of nitration products with significant abortion in the visible region. While there was little or no evidence for the formation of nitro products in the case of PG, we did find that PV forms nitro products which strongly absorb in the visible region with a maximum around 464 nm. In the second set of experiments, lysozyme was allowed to PN±CO_2 for the formation of

nitrolyzyme and then examined if either PG or PV interfere with this process. There was marginal inhibition of lysozyme nitration by PV but not by PG, although the latter was employed in mM concentrations. Although the results of the present study need further confirmation with product analysis, we believe that the effects of antibiotics on protein nitration may be less significant compared to N-acetyl-L-cysteine and other reactive thiol antioxidants.

2922 Dual Mechanisms Suppress Meloxicam Bioactivation Relative to Sudoxicam

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Thiazoles are biologically active aromatic heterocyclic rings occurring frequently in natural products and drugs. These molecules undergo typically harmless elimination; however, a hepatotoxic response can occur due to multistep bioactivation of the thiazole to generate a reactive thioamide. A basis for those differences in outcomes remains unknown. A textbook example is the high hepatotoxicity observed for sudoxicam in contrast to the relative safe use and marketability of meloxicam despite the difference in one methyl group. Both drugs undergo bioactivation and meloxicam possesses an additional detoxification pathway due to hydroxylation of the methyl group. We hypothesized that thiazole bioactivation efficiency is similar between sudoxicam and meloxicam due to the methyl group being a weak electron donator, and thus the relevance of bioactivation and likely toxicity, depends on the competing detoxification pathway. For a rapid analysis, we modeled epoxidation of sudoxicam derivatives to investigate the impact of substituents on thiazole bioactivation. As expected, electron donating groups increased the likelihood for epoxidation with a minimal effect for the methyl group, but model predictions did not scale well among types of substituents. Through novel analytical methods, we measured steady-state kinetics for metabolic bioactivation of sudoxicam and meloxicam by human liver microsomes. Sudoxicam bioactivation was ~7-fold more efficient than that for meloxicam, yet meloxicam showed an ~12.5-fold higher efficiency of detoxification than bioactivation. Taken together, sudoxicam bioactivation was 90-fold more likely than meloxicam considering all metabolic clearance pathways. Kinetic differences likely arise from different enzymes catalyzing respective metabolic pathways for sudoxicam compared to meloxicam based on phenotyping studies. Rather than simply an alternate detoxification pathway, the meloxicam methyl group suppressed the bioactivation reaction. These findings indicate the impact of thiazole substituents on bioactivation is likely more complex than previously thought and likely contributes to the unpredictability nature of their toxic potential.

2923 Using Activity-Based Labeling to Estimate Toluene Biodegradation Rates in Soils

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Toluene is a common organic contaminant in groundwater used for drinking water. Naturally-occurring bacteria use oxygenase enzymes to initiate aerobic toluene biodegradation. Currently, detection of aerobic toluene-oxidizing bacteria in groundwater or soils is typically established by measuring the abundance of the genes encoding these oxygenases. We hypothesize that more accurate estimates of the abundance of toluene-oxidizing bacteria (and their rate of toluene biodegradation) can be derived from methods that quantify active oxygenase abundance. The objective of this project is to develop an activity-based labeling (ABL) method to quantify catalytically-active forms of toluene-oxidizing oxygenases in soil bacteria. Our ABL method uses mechanism-based inactivator probes, click chemistry and fluors to covalently label the active site of toluene-oxidizing monooxygenases. The resulting fluorescent proteins are then quantified by near-infra-red scanning after separation of total cell proteins using SDS-PAGE. We have focused on a model toluene-oxidizing bacterium (*Burkholderia vietnamiensis* G4) and have identified 1,7-octadiyne [17OD] as the most effective ABL probe based on both rates of monooxygenase inactivation and fluorescent labeling specificity. The effects of 17OD are specific for toluene-2-monooxygenase based on substrate-dependent O_2-uptake assays and we have also established a limit of detection for free-living cells using this ABL approach. To extend these studies into soils, we have also developed a whole cell extraction method using Nycodenz density gradient centrifugation that is comparably effective to methods for direct extraction of proteins from soils samples. Our future goals are to confirm the identity of the fluorescently-labeled protein using proteomic analyses of in-gel digestions of these proteins. We also plan to compare toluene-oxidizing activity and ABL responses in toluene-stimulated and control soil microcosms, and compare these responses to toluene oxygenase gene abundance measurements made using qPCR. This ABL method is also applicable to many other oxygenases that, for instance, oxidize important emerging contaminants including 1,4-dioxane and 1,2,3-trichloropropane as well as other pollutants including chlorinated solvents and fuel oxygenates. We are currently exploring the use of the ABL method to evaluate natural attenuation in water samples from fields contaminated with chlorinated solvents.

2924 Investigating the Impact of Trisomy 21 on Xenobiotic Biotransformation Using Human Dermal Fibroblasts

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While Down syndrome (DS, Trisomy 21) is generally associated with intellectual deficits and distinct physical features, this population of individuals experiences a host of additional comorbidities including type 1 diabetes, obesity, leukemia, inflammation and Alzheimer's disease. These comorbidities are of great significance to this study due to the fact that genetics, disease states, and cofactor levels can greatly impact xenobiotic biotransformation. To assess the impact of trisomy 21 on xenobiotic biotransformation, we investigated drug metabolizing enzyme (DME) gene expression in age- and sex-matched pairs of human dermal fibroblasts. By using Phase 1 and Phase 2 qPCR arrays, we analyzed the basal expression of 84 Phase 1 and 84 Phase 2 DME genes between control (CTRL) and DS. We detected significant differences in the expression profiles of numerous cytochrome P450s (CYPs), alcohol dehydrogenases, aldehyde dehydrogenases, glutathione transferases (GST) and sulfotransferases. Fibroblasts were then exposed to benzo[a]pyrene (BaP) to stimulate the aryl hydrocarbon receptor (AhR) and downstream drug metabolizing enzyme (DME) gene expression was analyzed. After 24 hours of BaP (10μM) stimulation, we detected variations in DME gene expression in DS compared to CTRL with ALDH3A1 (2.5 to 2 fold change in CTRL vs DS), CYP1A1 (3.8 to 0.4) and CYP1B1 (4.0 to 2.5). These studies highlight the inherent differences that having the presence of trisomy 21 has on DME inducibility via the AhR. Further, these data show that DS individuals do not metabolize xenobiotics in a manner similar to euploid individuals, indicating that additional characterizations are necessary to effectively understand drug and toxicant metabolism in this unique genetic background. Future studies will investigate gene expression downstream of additional xenosensor transcriptional factors, like the constitutive androgen receptor (CAR) and the pregnane X receptor (PXR).

2925 Novel Role of the Ah Receptor (AhR) Ligand 6-Formyllindolo[3,2-b]Carbazole (FICZ) in Cytochrome P450 (CYP)1A Induction by Hyperoxia

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Supplemental oxygen administration is frequently encountered in infants and adults with pulmonary insufficiency. Hyperoxia causes lung injury in experimental animals, and contributes to bronchopulmonary dysplasia (BPD) in premature infants, and ARDS in adults. CYP1 enzymes have been implicated in oxygen injury. Hyperoxia is known to induce CYP1A enzymes but the mechanisms are not fully understood. In this study, we tested the hypothesis that FICZ, formed under hyperoxic conditions, is the novel endogenous ligand of the Ah receptor (AhR) that mediates induction of CYP1A enzymes in lung and liver *in vivo* by hyperoxia. Adult male (8-10 week-old) wild type (WT) (C57BL/6J) WT, Ah receptor (AhR)-null, *Cyp1a1*_null, or *Cyp1a2*_null mice were maintained in room air or exposed to hyperoxia (> 95%) for 6 h. In some experiments, WT mice were exposed to vehicle corn oil (CO) (20 μl/mouse) or FICZ dissolved in CO (2 mg/kg), i.p., once daily for 3 days, and were maintained in room air or exposed to >95% O_2. Levels of FICZ in lungs was determined by LC-MS/MS. Hyperoxic lung injury was assessed histologically and by determining lung weight/body weight (LW/BW) ratios. FICZ was detectable in WT mice under room air conditions and after hyperoxia for 6 h, the FICZ levels were significantly increased. The AhR-null mice showed complete suppression of FICZ. In *Cyp1a1*-null and *1a2*-null mice, FICZ levels were doubled in room air compared to WT mice. FICZ caused marked induction of hepatic and pulmonary CYP1A enzymes compared to control. WT mice, pre-treated with CO showed severe lung injury after hyperoxia for 72 h, but the FICZ-treated animals were less susceptible to hyperoxic lung injury. The increases in FICZ levels after hyperoxia suggests that oxygen induces CYP1 enzymes such as CYP1A1/1A2 through formation of FICZ, an endogenous ligand for the AhR. The increased levels of FICZ in *Cyp1a1*-null or *Cyp1a2*-null mice suggests that both these enzymes play a role in metabolizing FICZ. Future studies could lead to the development of novel endogenous non-toxic ligands of the AhR in the prevention and/or treatment of BPD and ARDS in humans.

2926 Toxicological Responses in the Tongue Sole *Cynoglossus senegalensis* Naturally Exposed to Contaminants at the Ilaje Coastal Area of Ondo State, Western Niger Delta, Nigeria

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We have investigated toxicological (biotransformation and oxidative stress) responses in relation to contaminant burden in *Cynoglossus senegalensis* from the Ilaje coast, that receives complex mixtures of untreated effluents and run offs from oil exploration activities. Adult male and female *C. senegalensis* (n=416) were collected from October 2015- March 2017 from Araromi (putative control site), Ayetoro, Obenla and Awoye along the Ilaje coast. Hepatic mRNA expression for *cyp*1a, *cyp*1b, *ugt*2a, *gpx* and *cat*, were analyzed by qPCR, while enzymatic assays for EROD, PROD, BROD, MROD, GST, GR, GPx and CAT were also analyzed using standard methods. In addition, protein expression for CYP1A and CYP3A were measured by western blot and biota concentrations of trace metals and polycyclic-aromatic hydrocarbons were analyzed. We observed site- and gender-specific significant biotransformation and oxidative stress responses that were influenced by sampling season. These changes at mRNA and functional (enzyme and protein) levels paralleled biota contaminant burden, particularly for PAHs. Our findings suggest that contaminants (PAHs and metals) at the Ilaje coastal area may be eliciting overt physiological and health effects in *Cynoglossus senegalensis* and other marine biota at this vulnerable marine ecosystem. These effects are further confounded by negative biological consequences with significant effects on the livelihood of humans who depend on these aquatic resources as a significant source of food through fisheries.

2927 The Safener Benoxacor Is Enantioselectively Metabolized by Sprague Dawley Rat Subcellular Fractions

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Safeners (e.g. dichloroacetamide safeners) are classified as inert ingredients by the Environmental Protection Agency, but exhibit biological activity by inducing metabolic enzymes in crops to protect them from herbicide damage. Due to their "inert" classification, they are often overlooked in the literature and only limited information about their metabolites and toxicity is available. One of the most commonly applied dichloroacetamide safeners is benoxacor. The goal of this study was to elucidate the enantioselective metabolism of benoxacor *in vitro*. We studied the depletion of benoxacor in microsomes (5 µM benoxacor, 0.1 mg/mL microsomal protein, 1 mM NADPH, 37°C) and cytosol (5 µM benoxacor, 1 mg/mL protein, 5 mM glutathione, 37°C) prepared from female and male rat livers. Benoxacor levels were determined by gas chromatography-mass spectrometry. Enantiomeric fractions (EF) were determined by enantioselective gas chromatography with electron capture detection. Benoxacor was enantioselectively metabolized in both microsomal and cytosolic incubations. After 30 minutes, 48% and 45% of benoxacor remained in incubations with microsomes and only 18% and 9% remained in incubations with cytosol of female and male rats, respectively. Microsomal incubations showed an enrichment of the benoxacor enantiomer eluting first on the enantioselective column (E_1-benoxacor). A more pronounced enrichment was noted in incubations with microsomes from female than male rats (EF = 0.67 vs. EF = 0.60, respectively). Incubations with cytosol from female rats resulted in a slight enrichment of E_1-benoxacor (EF = 0.54), while incubations with cytosol from male rats displayed an enrichment of the second eluting enantiomer (E_2-benoxacor; EF = 0.43). The current results indicate that benoxacor is enantioselectively metabolized by microsomal and cytosolic enzymes present in Sprague Dawley rat subcellular fractions, and further research is needed to determine the effects of enantioselective metabolism on the toxicity of benoxacor. *[CBET-1702610/1703796.]*

2928 Exploring the Effect of Genetic Polymorphisms on Predicted High-Performance Aircraft Pilot Exposures

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Previous work used high performance aircraft pilot exhaled breath samples with physiologically based pharmacokinetic (PBPK) modeling to predict in-flight concentration ranges for some possible inhalation exposures. These predictions, however, were point estimates for an "average" pilot. That work was expanded to include Monte Carlo (MC) analyses to account for interindividual variation, but did not account for complex differences in metabolism due to genetic polymorphisms that could affect the pharmacokinetics of potential exposure chemicals and, thus, the potential risk due to these exposures. Available data on known genetic polymorphisms for metabolism of volatile organic chemicals, such as frequency and impact of the polymorphism on metabolism, were compiled from the literature and analyzed as to their usefulness for predicting differences in potential exposure. Pertinent information was then incorporated into parameter distributions for use with the previously used PBPK model in MC analyses. These analyses included distributions for only the metabolism parameters and for all parameters to explore the impact of the genetic polymorphism in conjunction with individual variability. Where data were available for polymorphisms for metabolism of both the parent and metabolite, distributions for both were included in the MC analyses to assess the effect upon parent chemical predictions as well as the effect upon metabolite pharmacokinetics. Predicted area under the curve (AUC) for venous blood concentrations as well as distributions for exposure reconstructions were compared to the previously determined point estimates and exposure reconstructions to demonstrate the resulting impact of accounting for genetic polymorphisms. Differences in both predicted AUCs and exposure distributions were noted. In particular, the previous AUC estimate for simulated isopropanol exposure was below the mean AUC for 3 alcohol dehydrogenase genotypes and above for 3 others. This work demonstrates the importance of understanding the impact of genetics when assessing risk to protect a "population" as opposed to an "average" individual. The work presented here is part of an ongoing effort aimed at developing a capability to best assess true pilot physiology in a "virtual" context so that aircraft cabin exposure guidelines may be produced to ensure limited probability of contaminated cabin spaces.

2929 Predicting the Transfer of Chemicals through Lactation Using Quantitative Structure-Activity Relationship (QSAR) Modeling

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Chemicals are regularly detected in breast milk samples from the general population, indicating that children are exposed during lactation. However, the transfer of chemicals from maternal blood to breast milk is unknown for most chemicals on the market and their metabolites. In this study, we aimed to develop a QSAR model to predict milk-plasma concentration ratios for chemicals based on their chemical structural properties. We compiled published milk-plasma concentration ratios for 183 chemicals, including 110 pharmaceutical drugs and 72 environmental chemicals (e.g., polychlorinated biphenyls, dioxins/furans, organochlorine pesticides organochlorines, phenols, parabens, perfluoroalkyl substances, and several relevant metabolites). We then used the Mordred software to calculate 1614 chemical descriptors for each chemical in the database. Multiple predictive models (e.g., random forest, k-nearest neighbor, lasso regression) were developed through 10-fold cross-validation using 80% of the chemicals (internal dataset), and tested against the remaining 20% (external dataset) using the Sklearn package in Python. We estimated the performance of each model based on the coefficient of determination (R^2), cross-validation coefficient of determination (R^2_{cv}) and external validation coefficient of determination (R^2_{ext}). Milk-plasma concentration ratios ranged from 0.01 to 20.47, with a median value of 1.25. The random forest model yielded the best results in terms of R^2 (0.94), R^2_{cv} (0.52) and R^2_{ext} (0.50). Several descriptors contributed to the random forest model, including acidic and basic group counts. QSAR modeling will help estimate the lactational transfer for data-poor chemicals, namely through the parameterization of physiologically-based pharmacokinetic models of pregnancy and lactation.

2930 Human Duodenum Intestine-Chip Model to Study Drug-Induced Toxicity

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The damage of non-steroidal anti-inflammatory drugs (NSAIDs) on gastric and duodenal mucosa has gained much attention in the last few years. A large number of people take NSAIDs each day. This number has grown significantly with increasing use of over-the-counter and prescription NSAIDs. About 30-50% of NSAIDs users present endoscopic lesions, such as erosions and ulcerations, without clinical symptoms. A lack of reliable pre-clinical models to capture critical aspects of human intestinal physiology limits our ability to dissect the exact mechanisms driving NSAIDs-induced injury of the intestinal mucosa and enable improved risk assessment. Organs-on-Chips technology can be combined with intestinal organoids to overcome some of the limitations often associated with organoids such as architecture, ability for expansion to polarized monolayers, and sampling from the epithelial side. The Duodenum Intestine-Chip contains human primary intestinal organoids derived from duodenal biopsies, along with intestinal tissue-specific microvascular endothelial cells to emulate the complex dynamic state of the native human intestine required for normal cell architecture and function, including appropriate extracellular matrix, tissue-tissue interface, and the mechanical forces that recreate both intestinal peristalsis and blood flow. In this study, we used Duodenum Intestine-Chip to model indomethacin-induced injury of human intestinal tissue. Indomethacin showed to be toxic to duodenal epithelium when administered at the epithelial side. As a result, Duodenum Intestine-Chip exposure to indomethacin led to a loss of intestinal barrier function. Consistent with the fact that the intestinal barrier function is disrupted in 60-80% of patients using NSAIDs therapy. In addition, concentration-depended increase in the release of various injury markers, such as LDH, ROS and I-FAPB, was observed in Duodenum Intestine-Chip after 24 hours of exposure, in contrast to conventional organoids that appear to fail to recreate the concentration-dependent responses. In conclusion, Duodenum Intestine-Chip provides a human-relevant model for studies to determine drug-induced toxicity that could potentially be applied into pre-clinical testing of drug safety and efficacy.

2931 Quantitative Structure-Activity Relationship (QSAR) Modeling to Predict the Transfer of Chemicals across the Placenta

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Risk assessment of prenatal exposure to chemicals is paramount considering the potential adverse effects on the developing organism. To date, few tools are available to predict the placental transfer of chemicals across the placenta, especially for environmental chemicals. The aim of this study was to develop a QSAR model predicting cord-maternal plasma concentration ratios for environmental chemicals. We compiled cord-maternal plasma concentration ratios from published articles on multiple environmental chemicals including polychlorinated biphenyls, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and pesticides. We also included cord-maternal plasma concentration ratios for certain drugs to expand the domain of applicability. Only ratios from studies where cord and maternal blood samples were collected within 24 hours were kept in the dataset (n=88). We calculated 1614 descriptors for each chemical using the Mordred software. A suite of predictive models were developed through 10-fold cross-validation using 80% of the chemicals. Models were then tested against the remaining 20% of chemicals (external dataset). All statistical analyses were performed using the Sklearn package in Python. Cord-maternal plasma ratios ranged from 0.05 to 3.05 (median: 0.52), indicating that some chemicals preferentially partition into maternal or fetal plasma during pregnancy. The model developed using random forest displayed the greatest precision, with a coefficient of determination (R^2) of 0.98, a cross-validation R^2_{cv} of 0.89, and an external validation R^2_{ext} of 0.87. This level of precision was among the highest achieved for the placental transfer of chemicals and drugs. Using this QSAR model will help quantify fetal exposure to chemicals based on measured maternal plasma levels, or through the parameterization of physiologically-based pharmacokinetic models of pregnancy.

2932 Development and Application of an Interactive Physiologically Based Pharmacokinetic (iPBPK) Model Interface to Estimate Withdrawal Intervals for Penicillin G in Cattle and Swine

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Physiologically based pharmacokinetic (PBPK) models are a useful tool in the estimation of drug tissue residue depletion and withdrawal interval recommendations in food animals and they can be used to conduct extrapolation across species, ages, production classes, administration routes and doses. Traditional PBPK models are difficult to use by individuals with no modeling experience. Therefore, a user-friendly PBPK modeling framework and web-based interface would be beneficial. Penicillin G is a commonly used drug in food animals and was among the top three violative drug residues identified by the US National Residue Program from 2010-2018. The objective of this study was to develop a PBPK model for penicillin G in different production classes of cattle and swine and convert the model to a web-based user-friendly interactive PBPK (iPBPK) interface. Traditional PBPK models for penicillin G in market-age cattle and swine were developed and validated based on published data from the Food Animal Residue Avoidance Databank (FARAD). The model was then extrapolated to dairy cows and heavy sows. This traditional penicillin G model was converted to an iPBPK interface using R Shiny. These PBPK models can be used to predict withdrawal intervals of penicillin G after extralabel administration to cattle and swine across production classes, administration routes and doses in real time. The iPBPK framework presents proof-of-concept for developing web-based iPBPK interfaces for drug withdrawal interval estimations and animal-derived food safety assessment.

2933 Apparent Permeability Coefficients to Parameterize PBPK Models for Inhalation: Impact of the *In Vitro* Setup

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This study aimed at the determination of apparent permeability coefficients (P_{app}) of the inhalable antibiotic ciprofloxacin hydrochloride monohydrate (CHM) in human lung barrier models and to utilize these coefficients as input parameters for a lung PBPK model (physiologically based pharmacokinetic model). Two different cell models were used resembling different lung regions: The Calu-3 cell line expressing features of differentiated small airway epithelial cells as well as functionally immortalized human alveolar epithelial (CI-hAELVi) cells. For both cell models, P_{app} values were measured in a submerged setting and under air-liquid interface (ALI) exposure conditions. For submerged exposures, the antibiotic CHM was dissolved in culture medium and added to the apical compartment. ALI exposures were done with CHM aerosol generated using the PreciseInhale™ device. For efficient and precise aerosol exposures, the P.R.I.T.®ExpoCube® was used. CHM concentrations in the different compartments were analysed by LC-MS/MS. In the case of ALI exposures, the CHM mass deposited on the apical cell surface had to be converted to concentration values. This was done by assuming a thickness of 2 µm for the lining fluid layer for Calu-3 and AT-1 cells and dissolving of CHM in the resulting volume. The resulting P_{app} coefficients were similar for both cell models, the levels being $1.99*10^{-8}$ cm/sec for AT-1 and $1.09*10^{-8}$ cm/sec for Calu-3, respectively. The cell models under submerged conditions showed higher P_{app} values of $6.34*10^{-7}$ cm/sec for AT 1 cells and $7.24*10^{-7}$ cm/sec for Calu-3 cells. P_{app} values obtained from both *in vitro* settings were used as input parameters for the physiologically based pharmacokinetic (PBPK) model to simulate the absorption and distribution processes in the human body. Resulting peak blood concentrations for ciprofloxacin were 5 fold higher when P_{app} coefficients obtained from submerged experiments were used. However, comparison with human literature data revealed that simulations based on P_{app} coefficients calculated for ALI exposures produced blood levels that were very close to the human *in vivo* situation. These findings suggest that the experimental setup is highly important when *in vitro* data are to be used as input parameters for PBPK models to predict the bioavailable dose after inhalation exposure in humans.

2934 Evaluation of GastroPlus Software for Predicting Toxicokinetic Parameters of Agrochemicals following Repeated-Dose Dietary Feeding

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The use of *in silico* approaches for assessing the risks of chemicals in a more mechanistic and high throughput manner has become increasingly important to support human health risk assessment. Current approaches rely primarily on *in vivo* testing following intake of compounds administered by different routes and intake patterns across a day. Physiologically based pharmacokinetic (PBPK) modeling, coupled with *in silico*- and *in vitro*-derived chemical-specific parameters, provides an effective framework for improving quantitative *in vitro*-to-*in vivo* extrapolation (IVIVE). The combination of *in silico*- and *in vitro* parameter estimation with PBPK modeling can then be used to predict the *in vivo* absorption, distribution, metabolism and excretion. Current *in silico* modeling programs are not user-friendly or require extensive training to use. For example, TK modeler, a Microsoft Excel-based pharmacokinetic modeling program, can model single, multiple bolus or dietary dosing but it lacks PBPK model compartments, which are parameterized using known physiology that correspond to different organs or tissues in the body. In this research, GastroPlus™ software was evaluated to compare observed *in vivo* data with simulated results for two molecules: Penoxsulam and X11719474 (a sulfoxaflor metabolite) following repeated dose feeding in rats. These molecules were selected due to simplicity of metabolism pathway, rapid and almost complete absorption, little to no metabolism in an *in vitro* hepatocyte clearance assay, or elimination as largely unchanged parent compound following oral administration. A diurnal feeding pattern was used to model the dietary intake of the test substances and predict systemic exposure during 28 or 90-day toxicity studies. Predicted plasma concentrations and corresponding AUC values were improved by incorporation of physicochemical (i.e. LogD/P, water solubility and pH) and/or *in vitro* ADME (i.e. intrinsic clearance in primary hepatocytes and plasma protein binding) properties. Current predictions are 1 to 3-fold above the measured *in vivo* plasma concentrations. Based on this evaluation, chemical-specific parameters were critical to improving model implementation. Overall, the results demonstrated that GastroPlus™ can be used to predict toxicokinetic parameters in rats for poorly metabolized chemicals following repeated dose feeding. Simulation with other compounds is in progress to validate this approach.

2935 Development and Systematic Comparison of Pregnancy Physiologically Based Toxicokinetic Models for Bisphenols A and S

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Physiologically based toxicokinetic (PBTK) models aid in the process of health risk assessment by incorporating *in vivo* and *in vitro* toxicokinetic data, known physiological parameters, such as fractional blood flows and organ volumes, and *in silico* tools in order to make predictions about the extent of chemical exposures. These models are particularly important in understanding exposures during vulnerable periods of life such as pregnancy and *in utero* development. Bisphenols A and S (BPA and BPS) are the first and second most abundant bisphenols detected in human blood and urine in the US. Herein, we aimed to develop physiologically sound pregnancy PBTK (P-PBTK) models for BPA and BPS using several paired maternal and fetal pregnancy toxicokinetic datasets that include parent and metabolite concentrations of BPA and BPS in sheep plasma, as well as two administration routes. Following sensitivity analysis and extensive optimization, we determined that different values of parameters produce equally good fits to the calibration datasets, for both models. After exclusion of all physiologically implausible models we were left with a set of models with narrow ranges for all estimated parameters. Among these, we picked two representative models, one for BPA and another for BPS. Both models fit most endpoints of the available datasets within one standard deviation of individual data points. The developed models provide insight into toxicokinetics of the parent compound and its metabolite in maternal and fetal tissues of interest (e.g. fetal brain), as well as gestational age, species, dose and route of exposure extrapolation. In addition, the analysis provides estimates of parameters that are difficult to measure experimentally, such as bioavailability of subcutaneous absorption and deconjugation in the fetal liver. To the best of our knowledge these are among the first P-PBTK models for BPA and BPS in sheep. Both models advance our understanding of bisphenol toxicokinetics and can assist the development of future P-PBTK models for related chemicals. Additionally, we make recommendations for future experimental toxicokinetic study designs to facilitate the construction of mechanistically sound P-PBTK models with greater translational relevance. *JG was supported by NICHD T32HD087166. Supported by NIEHS R01ES027863 to AVL.*

2936 INOS-Independent SNO Generation at Low Ph and SNO Prediction Using a Neural Network

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Nitrosylation is a form of post-translational modification, which provides modularity to protein function after translation, allowing cells to adapt to a variety of stimuli. However, unlike more "canonical" post-translational modifications, the motif and environmental conditions under which cysteine residues are nitrosylated are unclear. The chemistry of formation presupposes that there may be subsets of cysteines that are nitrosylated via different mechanisms. The most comprehensive database for SNO, dbSNO 2.0, indiscriminately collected literature reports for nitrosylation and thus cysteines do not follow a consistent set of environmental conditions and represent different populations of SNO. We hypothesize that different SNO populations may be generated by altering microenvironment of the cysteine residues. SNO protein formation was examined in RAW cells with and without LPS stimulation, while altering the intracellular environment using mitochondrial poisons (MP). Cells were treated with oligomycin, FCCP, or rotenone after 1 hour of stimulation with LPS. Treatment significantly increased nitrite release to the media. Both rotenone, the complex I inhibitor, and oligomycin, the ATP synthase inhibitor, significantly reduced nitrite production. FCCP did not alter nitrite production. Western blot of cell lysate confirms iNOS in LPS-stimulated cells for all treatments, with reduced banding in MP. Using Biotin Switch, we were able to show significant production of SNO proteins in response to LPS, which was unaltered by FCCP. However, rotenone completely abrogated SNO formation, while oligomycin forms SNO even in the absence of LPS. These studies indicate that nitrosylation is influenced by the intracellular environment, with cellular acidification being a major mechanism. Identification of the SNO-protein identities within cell lysate will allow one to create database for training a neural network for cysteines. To demonstrate viability of a neural network approach, an algorithm was trained on a subset of dbSNO entries, randomly selecting 20% of the trimmed sample as the validation set. A window size of 19 was discovered to return the optimal MCC for the following hyperparameters: two hidden layers, 50 neurons each layer, learning rate of 0.0005, L1 Regularization parameter of 5, and 2000 iterations. Sigmoid activation function was used for the hidden layers, TanH activation function for the output layer, and a quadratic cost function to backpropagate. A median MCC of 0.230, with a sensitivity of 0.661, a specificity of 0.569, a positive predictive value of 0.589, and a negative predictive value of 0.642 was obtained. *NIH ES005022 HL086621.*

2937 Use of a Weighted Scheme for the Interpretation and Contextualization of *In Vitro*- and *In Silico*-Derived Estrogenic Endpoints

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With the rise of interest in endocrine disruption by the scientific community and the general public, a clear and scientifically sound approach to evaluating potential endocrine disrupting properties is critical. Advances in *in vitro* and high throughput technologies have provided a wealth of data which provide insight into the EATS (endocrine, androgen, thyroid, steroidogenesis) modalities. A current regulatory challenge is the utilization of multiple data streams to arrive at a conclusion regarding endocrine activity. An additional challenge is that many chemicals have not been tested using existing *in vitro* approaches, thus there is a need for additional tools to provide information regarding endocrine disrupting properties. *In silico* modeling offers a robust, widely-applicable, cost- and time-effective approach to fill these data gaps. This work set out to place the development of an *in silico* predictive model into the relevant biological context to ensure the appropriate interpretation of model outputs and builds off an accompanying presentation which highlights the technical robustness of 18 models developed to predict endocrine bioactivity. Of the 18 ToxCast assays for estrogenicity, this work focuses on the five assays that describe transcription and protein production and weighs less heavily those assays that describe earlier aspects of the adverse outcome pathway, such as receptor binding. This weighting approach was validated by a selection of reference chemicals designated by the EPA and derived from *in vivo* and *in vitro* studies. Compared to the EPA *in vivo* reference chemicals, the weighted scheme of *in silico* model outputs predicts with 93% concordance. An alternate aggregation approach has gained wide acceptance in the regulatory community, but lacks the ability to evaluate novel chemicals; the approach described herein is thus advantageous in that it can predict activity for chemicals that are not already associated with *in vitro* data. This research provides the incorporation of adverse outcome pathway methodologies into the interpretation/aggregation of *in vitro* and *in silico* data sources and can

be used to predict estrogen bioactivity for novel chemicals. Such frameworks are useful for the condensation of many datapoints into a single output or conclusion for regulatory decision-making purposes.

2938 Utilization of GastroPlus Physiologically Based Pharmacokinetic (PBPK) Modeling in Nonpharmaceutical IVIVE Predictions

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As regulatory toxicology transitions to non-animal methods, there is a need to develop and validate *in vitro*-to-*in vivo* extrapolation (IVIVE) methods. Further, there is a recognized need to improve agricultural/industrial chemical PBPK modeling, and previous work has shown that chemical-specific *in vitro* PBPK parameters can improve PBPK predictions. In the current study, GastroPlus™ PBPK predictions were generated for four compounds - myclobutanil, oxyfluorfen, pronamide, and tricyclazole. New data were generated for the main PBPK parameters (i.e., caco-2 permeability, plasma binding, blood to plasma ratio, and rat liver microsomal half-life) and integrated into the PBPK models containing 12 organ compartments and compared using GastroPlus™ software. The fold-error (FE) of the predicted plasma AUC (area under curve) of parent pronamide without *in vitro* PBPK parameters compared with published plasma AUC values was in the range of 2.7 to 7.8; however, the FE was reduced to 1.2 to 4.2 by incorporating data for *in vitro* PBPK parameters into the model. Furthermore, the predicted plasma time course and the steady-state plasma concentration (Css) correlated well with published data when *in vitro* PBPK parameters were included in the model. In contrast, *in vivo* total radioactivity-based plasma concentrations (pronamide plus it's metabolites) had limited applicability as FEs ranged from 11 to 38 even with *in vitro* PBPK parameters applied to the model. In addition, the parent-based tissue concentrations of these four agrochemicals were predicted in rats and humans after single or 30-day repeat exposure to 10 mg/kg/day. These data indicate that GastroPlus™ PBPK predictions can be improved with chemical-specific *in vitro* PBPK parameters, and, in the cases examined, are applicable for IVIVE estimations.

2939 Evaluation of a Rapid, Multi-chemical Human Gestational Physiologically Based Toxicokinetic Model

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Human health chemical risk assessment involves consideration of potentially susceptible populations, including pregnant women with developing fetuses. It is estimated that humans encounter thousands of artificial chemicals in their environments, few of which have been characterized in terms of health risk. To assess any risk posed by a chemical, toxicokinetic (TK) information is needed to relate chemical exposure to potentially toxic tissue concentrations. As human-specific gestational exposure data are unavailable for most chemicals, physiologically-based TK (PBTK) models are needed to extrapolate knowledge from existing *in vivo* toxicological studies and/or *in vitro* bioactivity data to human pregnancy. Development of chemical-specific PBTK models is resource-intensive. As an alternative, generic PBTK approaches in which a system of ordinary differential equations (ODEs) describes a standardized physiology and chemicals are characterized with a set of standard physical and *in vitro*-measured descriptors enables expedited, high-throughput modeling and prioritization. Here we report the systematic evaluation of a generic PBTK model of a human mother and a developing fetus. Building upon a statistically plausible set of parameters with which to simulate the major physiological changes that accompany pregnancy, as well as upon the existing high-throughput toxicokinetics (httk) software package in R, we present a generic tool for estimating the disposition of many chemicals throughout a maternofetal system. In the limit of "steady state" the model assumes that the ratio of the maternal to fetal plasma chemical concentrations is a function of the relative partitioning into the placenta, which may not vary for many chemicals with exception for some ionizable compounds and chemicals that cannot reasonably reach steady-state. We compare an analytic determination of the equilibrium ratio for 25 chemicals in maternal and fetal plasma to existing measurement data on maternal-to-umbilical plasma concentration ratios at delivery. The analysis stands to be extended to the over 944 already parameterized for this model in httk. Chemical membership spans classes from organochlorine pesticides to perfluorinated compounds. With sufficient evaluation, this gestational model may allow *in vitro-in vivo* extrapolation of reproductive toxicity-relevant bioactivity to maternal chemical exposures.

2940 Physiologically Based Pharmacokinetic Modeling and Simulation of Nanoparticle Delivery to Tumors in Mice

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Many studies have engineered nanomaterials with different physicochemical properties to increase the delivery efficiency to solid tumors, but the mean and median delivery efficiencies are only 1.5% and 0.7% of the injected dose (%ID), respectively according to a study using a non-physiologically based approach based on published data from 2005 to 2015. In the study, we applied a physiologically based pharmacokinetic (PBPK) modeling approach to analyze 376 datasets published from 2005 to 2018, and found mean and median delivery efficiencies at the last sampling time point of 2.22% and 0.75%ID, respectively. Also, the mean and median delivery efficiencies were 2.24% and 0.76%ID at 24 h, and were reduced to only 1.23% and 0.34%ID at 168 h, respectively after intravenous injection. Although these delivery efficiencies seem to be larger than previous findings, they are still low and represent a critical barrier in the clinical translation of nanomedicines. We explore the potential causes of the low delivery efficiency from a PBPK perspective. We also propose a long-term strategy to overcome this fundamental barrier with a focus on the role of PBPK modeling and simulations.

2941 Modeling Deposition and Uptake of an Inhaled Puff Emitted from an Electronic Nicotine Delivery System (ENDS) in the Human Respiratory Tract

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A puff from an electronic nicotine delivery system (ENDS) contains multi-constituent aerosols and vapors. The mixture is highly unstable and undergoes rapid thermodynamic changes. As a result, there will be significant changes in the characteristics of inhaled aerosols and vapors immediately after generation in the ENDS and while traveling through the respiratory tract. These changes need to be studied and accounted for when developing a dosimetry model to assess both vapor uptake and aerosol deposition. Hence, a coupled vapor uptake and aerosol deposition model was developed for a puff made up of water, glycerin, nicotine, propylene glycol, and flavors. The deposition model included the coagulation of airborne materials at high aerosol number concentrations and vapor exchange between liquid (aerosol) and vapor phases. The model accounted for the mixing of the puff with the dilution air at the end of mouth hold and reserve air when the puff reached the pulmonary space. The fate of all constituents in the puff was obtained at different sites of the respiratory tract. Overall, about 90% of the nicotine and propylene glycol were taken up by lung tissues whereas glycerin deposition was under 60%. Tissue dose from droplet deposition was higher than that for vapor uptake for all constituents of the puff. A software tool was developed based on the deposition model, which allows the users to study the fate of the inhaled puff under various ENDS use scenarios. The model is a powerful tool to inform the exposure and health risk evaluation of ENDS use. *This study was funded by the US FDA. This information is not a formal dissemination of information by FDA and does not represent Agency position or policy.*

2942 Generalized PBPK Model for Evaluation of Inhalation Exposure of Volatile Organic Compounds

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PBPK modeling is a valuable tool to evaluate inhalation exposure of volatile organic compounds (VOCs). However, many models employed in literature are ad hoc and built for a single purpose or molecule. These models generally look at a subset of major tissues and rely on experimental or fitted partition coefficients. A desirable approach would be to use a standardized PBPK model to predict exposure of VOCs rather than building ad hoc models. GastroPlus® has a physiologically based inhalation model that predicts the exposure in animals and humans for solution and dry powder doses. The goal of this work was to add a dosing route for VOCs and predict both uptake and clearance due inhalation and exhalation. The partitioning of the volatile compound between the vapor phase and the mucosal lining fluid in the lung is based on Henry's Law. Once partitioned into the mucus, the drug then diffuses though the epithelial cells to reach systemic circulation. The thickness of the mucus and epithelial cell layer is location dependent in the lung. Once VOCs have been absorbed into systemic circulation, GastroPlus has a full PBPK model to predict the systemic toxicokinetics which allows a variety of op-

tions for predicting tissue partition coefficients as well as elimination (linear clearance, non-linear metabolism, renal clearance, and metabolite tracking). Availability of physiologies for human and for animals commonly used in toxicological testing simplifies interspecies scaling and predictions of toxicokinetics in human. The VOC inhalation model was tested on several available literature datasets including ethanol, methanol, acetone, benzene, toluene, and xylene. Results show that the model does a good job at extrapolating systemic exposure from rat to human in almost all cases with the exception of ethanol where pulmonary metabolism had to be considered to fit human data; however, this is in accordance with other literature PBPK models. The average and maximum prediction error for Cmax across all compounds and datasets is 12.5 and 39.1% for rat and human. In summary, a new physiologically based VOC inhalation model has been added to the GastroPlus pulmonary module that allows prediction of toxicokinetics and interspecies scaling of exposure from animal models like rat to human. We believe this provides an improvement on current ad hoc or open source tools due to the volume of validation work on drug-like molecules in the GastroPlus platform.

2943 Physiological Parameter Values for Physiologically Based Pharmacokinetic Models in Food-Producing Animals. Part I: Cattle and Swine

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Physiologically based pharmacokinetic (PBPK) models of chemicals in food animals are a useful tool in estimating depletion of chemical tissue residues and withdrawal interval recommendations. Physiological parameters such as cardiac output, organ weights and blood flows are an important component of a PBPK model. The objective of this study was to critically evaluate published PBPK-related physiological parameter data in food animals, including cattle, swine, goats, sheep, chickens, and turkeys. Literature searches were performed in PubMed and Google Scholar. Relevant literature was reviewed and tables of cardiac output, relative organ weights (% of body weight) and relative blood flows (% of cardiac output) were compiled for different production classes of cattle and swine as Part I of this series of study. The mean and standard deviation of each parameter were calculated to conduct population PBPK analysis. The results showed that the cardiac outputs were 9.09 ± 2.77, 5.45 ± 1.47, 8.70 ± 1.62 in unanesthetized calves (≤10 months old), cattle (>10 months old), and market-age swine, respectively. In cattle, the relative organ weights were 36.10 ± 11.73, 1.23 ± 0.21, 0.21 ± 0.04, and 12.27 ± 5.21 in muscle, liver, kidney, and adipose tissue, respectively. In swine, these values were 36.32 ± 2.66, 2.04 ± 0.33, 0.37 ± 0.11, and 15.44 ± 2.65, respectively. These compiled data provide a comprehensive physiological parameter database for developing PBPK models of chemicals in cattle and swine to help animal-derived food safety assessment. This work also serves a basis to compile data in other food animal species.

2944 Physiologically Based Prediction of the Impact of Nonchemical Stressors in the Aviation Environment on Xenobiotic Dosimetry in Humans: Effect of Barometric Pressure or Altitude

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Aircraft can be a physiologically challenging environment due to hypobaria, acceleration, low humidity, thermal variation, vibration, and other factors. The special characteristics of the military aviation operational environment and their combined impact on air crews constitute a challenge to the health risk assessment strategies used to identify situations where risk management action is needed. Among the strategies available for the quantitative comparison of risks between the general population and populations with distinctive health concerns or that experience particular environmental stressors is physiologically based pharmacokinetic (PBPK) modeling. PBPK models facilitate these comparisons by prediction of how target-tissue specific doses are altered when a stressor, such as high altitude, effects changes in physiological parameters. Cardiac output, regional blood flow, and alveolar ventilation rate for personnel acutely exposed to altitude ranging from moderate to extremely high were extracted from published data from 51 groups of human subjects and described mathematically via regression models. A scenario where a pilot could inhale organic compounds in-flight during routine training was simulated for three test chemicals. When exposed at the recommended Threshold Limit Values, arterial blood concentrations were predicted to be higher for exposure at 15,000 ft than at sea level. For example, during a series of two 1-h flights separated by a 2-h break, the peak blood toluene level was predicted to increase by 42% as compared to exposure on the ground. The predicted differences were greater for the two test compounds with higher blood: air and fat: blood partition coefficients (toluene and 1,2,4-trimethylbenzene) than the less lipophilic cyclohexane. In summary, quantitative approaches to internal dosimetry prediction that take advantage of existing knowledge of physiological changes induced by occupational stressors have potential as tools in performing human health risk assessment.

2945 Development and Evaluation of a PBTK Model for Naphthalene for Use in Human Health Risk Assessment

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Naphthalene, a volatile organic compound found in moth repellants and petroleum-based fuels, has been shown to induce toxicity in the nasal epithelium of both mice and rats during chronic inhalation exposures. Any human health risk assessment for inhaled naphthalene would require the estimation of a human equivalent concentration (HEC), which is an inhaled air concentration that would produce a human internal dose equal to that occurring in an animal at a point-of-departure (POD) exposure concentration. One can estimate an HEC from an animal POD using a physiologically based toxicokinetic (PBTK) model. Confidence in an HEC estimated in this way increases if the model has been validated using both animal and human time-course internal concentration data. A published inhalation PBTK model for naphthalene was previously shown to predict rodent kinetic data well, so we sought to evaluate this model using human kinetic data. The most reliable human kinetic data available, however, were recorded during a ten-subject controlled skin exposure study and the PBTK model in question does not include a skin exposure route; therefore, we extended the model by incorporating skin compartments representing the stratum corneum (SC) and the viable epidermis. Adding these compartments required the introduction of two model parameters for which published values could not be found: diffusivity of naphthalene across the SC and the SC-to-exposure-vehicle partition coefficient. Furthermore, the human data revealed "background" blood concentrations of naphthalene in the subjects prior to exposure, so we introduced an intravenous dose rate parameter to account for baseline levels of napthalene. We calibrated these three new parameters in the modified PBTK model using data from the ten subjects of the controlled skin exposure study while maintaining the values for all other human parameters, including metabolic parameters, that were proposed for the original PBTK model. In this way, we were able to achieve reasonable agreement between the model predictions and kinetic data for all subjects. Our evaluation of the modified PBTK model using human data showed that this model can accurately predict internal doses of naphthalene and is thus a viable tool for estimating HECs for naphthalene human health risk assessments.

2946 Exploring the Application of Physiologically Based Pharmacokinetic Models in Acute Chemical Incidents: The National Toxic Substance Incidents Program

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Chemical release incidents in the United States include a variety of different chemicals that can have negative effects on nearby communities. The Agency for Toxic Substances and Disease Registry (ATSDR) historically tracked these chemical releases from 1991-2014 in up to 16 states with the Hazardous Substances Emergency Events Surveillance (HSEES) and the National Toxic Substance Incidents Program (NTSIP) systems. With the help of surveillance data, patterns of these different chemical releases can be studied with the intent of constructing a health-protective course of action. Physiologically-Based Pharmacokinetic models (PBPK) can be utilized to simulate the chemical exposures during an acute chemical incident. We have conducted a retrospective study of one such acute chemical release occurring in 2012 with the intent of examining the components needed to integrate PBPK-modeled exposure assessments in ATSDR's Assessment of Chemical Exposure (ACE) program. We examined data from a Vinyl chloride (VC) exposure investigation to explore the utility of PBPK to assess exposures in residential populations near the release site. At the release site, immediate estimates from real-time air monitoring indicated that air levels greatly exceeded Acute Exposure Guideline Levels (AEGL) of 1,200 ppm; the corresponding PBPK modeled VC blood levels of 3.17 mg/L. "Real time" and "after action" air modeling estimated VC levels at various distances from the release site at different time

points; PBPK modeling using those air VC levels suggested elevated blood levels in community residents in areas adjacent to the release site. The PBPK modeling also provided some temporal insight on residential blood levels of VC as the event played out over several days. These results indicate that PBPK may be a useful tool for reconstructing exposures associated with acute chemical releases. *The findings and conclusions in this presentation have not been formally disseminated by [the Centers for Disease Control and Prevention/ the Agency for Toxic Substances and Disease Registry] and should not be construed to represent any agency determination or policy.*

PS 2947 Liter-Equivalence Extrapolation for Four Trihalomethanes (THMs): What Drink Would It Take to Get the Same Internal Dose?

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Due to their presence in water as volatile disinfection byproducts, THMs pose a potential health risk from exposure via oral, dermal and inhalation routes. Environmental exposure studies demonstrate that dermal and inhalation exposure to water containing THMs during showering or bathing results in more THMs being delivered to the systemic circulation than oral exposure. Using a physiologically-based pharmacokinetic (PBPK) model, we conducted a liter-equivalence analysis (Leq). We determined the concentration in one liter of water consumed orally needed to achieve the same internal dose as from a 10-minute shower for chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM). Model structure and physiological parameters are the same as our published adult human BDCM model (Kenyon et al., 2016). Because human chemical-specific metabolism parameters are not available for all four THMs, rat metabolism parameters obtained from the literature were used to avoid confounding of comparisons across chemicals. For this same reason, human blood:air and rat tissue:air partition coefficients were used for all four THMs. We simulated showering for 10 minutes with water containing 8.2, 12.2, 13.5, and 8.7 µg /L for TCM, BDCM, DBCM and TBM, respectively. These are measured drinking water concentrations from a system with predominantly brominated species of THMs (Gulf coast TX, Lynberg et al., 2001). Two measures of internal dose, area under curve in venous blood (AUCv) and amount of specific THM metabolized in liver (AML) were evaluated. For AUCv, the oral Leq concentrations for the showering scenario were 20.9, 38.5, 43.8, and 29.9 µg/L for TCM, BDCM, DBCM and TBM, respectively. For AML, the oral Leq concentrations for the showering scenario were 1.3, 1.9, 1.9, and 1.4 µg/L for AML for TCM, BDCM, DBCM and TBM, respectively. These results demonstrate that dermal and inhalation exposure routes contribute significantly to internal doses of THMs reaching the systemic circulation (AUCv). In sum, consideration of the contribution of multiple routes of exposure to internal dosimetry should decrease uncertainty in dose-response characterization for water-borne THMs. *This abstract does not reflect US EPA Agency policy.*

PS 2948 An Investigation of Mirtazapine Pharamacokinetics in Cats Using Physiologic-Based Pharmacokinetic (PBPK) Modeling

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The study of drug dosing and metabolic effects in cats has been under-served by a relative lack of physiologic and pharmacologic data in this species, and feline-based PBPK models have not previously been represented in the literature. Further, known genetic abnormalities in cats (e.g., UGT1A6 and ABCG2, from which alterations in glucuronidation and drug transport arise) can often lead to severe drug toxicities in this population. Thus, an unfulfilled need exists for feline-specific PBPK models to investigate the dosing and pharmacokinetic profiles of therapeutic drugs used for treating feline disease. Mirtazapine (MTZ), a noradrenergic and specific serotonergic antidepressant, was selected for study as it is commonly used in veterinary medicine as a treatment for nausea and vomiting associated with chemotherapy, pancreatitis, and chronic kidney disease in cats. An investigation of mirtazapine pharmacokinetics (PK) in cats was conducted using an eight-compartment oral dosing PBPK model, developed using MCSim, and informed by available anatomic and physicochemical data for relevant feline target tissues. Although mirtazapine exhibits linear PK in humans, a prior feline-based clinical PK study demonstrated evidence of nonlinear PK in cats, thus analytical approaches to further characterize this behavior were explored via the PBPK model. The model was parameterized using Bayesian inference via a Markov chain Monte Carlo (MCMC) methodology and population pharmacokinetics were realized using Monte Carlo simulations. Results from a typical individual simulation of 1.88mg and 3.75mg oral mirtazapine doses yielded a dose prediction curve with a Cmax=63.4 ng/ml at a tmax=1.5 hrs and 126.4 ng/ml at a tmax=1.5 hrs for the respective doses, which compares favorably to results obtained from an *in vivo* study of orally dosed mirtazapine in cats (Cmax and tmax values of 73.1 ± 45.5 ng/ml and 1.6 ± 1.3 hr, and 156.5 ± 92.4 ng/ml and 1.5 ± 1.4 hr, for the analogous doses, respectively). Additionally, Monte Carlo simulations provided prediction intervals that bounded the broad majority of available experimental data points. The results from this study further build upon the developmental computational framework for investigating drug disposition in cats, with a view toward enabling a predictive approach for better informing feline drug dosing protocols, especially for cases where drug disposition may be impacted, such as in chronic renal failure or liver disease.

PS 2949 Advantage of Read-Across and Pharmacokinetics Modeling for the Exposure Assessment of Persistent Organic Pollutants

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Persistent organic pollutants (POPs) are highly resistant to degradation in the environment, bio-accumulate in living organisms, and can cause adverse effects. This class of chemicals include chlorinated POPs such as organochlorine pesticides, polychlorinated biphenyls, and dioxins. In this case study, we tested the hypothesis that an adequately developed, parameterized and validated human pharmacokinetic (PK) model describing a source chemical (i.e. the chemical with an existing human PK model) can be used to simulate PK data for a target chemical (i.e. the chemical with no existing human PK model). Potential single chemical surrogates for each of the organochlorinated POPs classes using analog similarity were identified. A concentration- and age-dependent dioxin pharmacokinetic model was parameterized for surrogate chemicals and coded using Berkeley Madonna software. Based on physiochemical and absorption, distribution, metabolism, and excretion (ADME) properties describing dioxin; similar chlorinated analogs were hexachlorobenzene, DDT/DDE and PCB 153. A series of PK models were parameterized to examine the utility for the surrogate chemicals. Overall, there was good agreement between the simulated and measured values. The models allow the estimation of hexachlorobenzene, DDT/DDE and PCB 153 concentrations for different exposure scenarios. Also presented is an application of the DDT/DDE model for comparison with biomonitoring data from the National Health and Nutritional Examination Survey. Hence, this series of PK models may be useful for interpreting human biomonitoring data as a part of an overall POPs risk assessment. *The findings and conclusions in this presentation have not been formally disseminated by [the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry] and should not be construed to represent any agency determination or policy.*

PS 2950 All Clinical Signs Are Important, but Some Are More Important Than Others: A Study on Associations Between Clinical Signs and Pathological Findings in Toxicity Testing

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Animal testing is a legal requirement for marketing of chemicals and pharmaceuticals. These experiments must take the 3R principles (Replacement, Reduction and Refinement) into consideration and clinical signs are generally used to monitor animal welfare during ongoing studies. In this study we investigate possible associations between clinical signs, body weight change and histopathological findings observed after necropsy. It was hypothesized that clinical signs and body weight loss observed during experiments could be used as early unspecific markers of organ toxicity. Data from three sequential toxicity studies in rats were analysed using the multivariate data analysis method partial least squares (PLS). Associations were found between the increased occurrence of clinical signs and histopathological findings in the thymus, testes, epididymides and bone marrow. The derived models predicted accurately over 80% of the animals' pathological findings, when building the model using all clinical signs available. The three most important clinical signs were piloerection, stained nostrils and decreased motor activity. A 5% body weight loss was found to be a strong empirical predictor of pathological findings. In conclusion, clinical signs and body weight loss constitute early and easy to observe markers of toxicity, with empirical predictive power for pathological findings. Thus, we recommend these signs to be utilised as informative endpoints for predicting toxicity in rats, as well as a non-invasive surveillance tool for animal welfare and toxicity assessment during *in vivo* studies.

2951 Benchmark Dose Software for Toxicological Risk Assessment in Japan

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For toxicological risk assessment, it is fruitful to have a user-friendly software for benchmark dose (BMD) modeling, possibly with graphical user interface (GUI). Our study aimed to devise an independent software equipped with BMD method. Using statistical package R, we drafted a modeling code for quantal response data based on animal experiment. The software uses a unique package OptimCL which implements parallel computation using a package snow and helps explore likelihood surface. Results: Due to the original package, modeling results are independent of initial value for unknown parameters. In addition to comparison of penalized likelihood and lowest limit value, BMDL, the software yields model averaging results as well as the averaging of three best models, the latter we believe as yielding the most reasonable BMDL value. A GUI-based software was devised without difficulty in specifying initial values for maximum likelihood estimation. The programmed software also offers the possible best option to be used for the point of departure.

2952 A Biologically Based Model to Quantitatively Assess the Impact of Chemical Exposure on Developing Hepatic Steatosis

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Fatty liver disease affects nearly one-third of the US population and hepatic steatosis, or excessive lipid accumulation in the hepatocyte, is the core manifestation of this ailment. Hepatic steatosis is a dysfunction of lipid homeostasis that is maintained through a complex network of biological events that include hepatic fatty acid (FA) uptake, *de novo* FA and lipid synthesis, FA oxidation, and lipid efflux. Hepatic FA *de novo* synthesis and uptake are mediated through the liver X (LXR), and pregnane X (PXR) receptors; respectively. Chemical binding to LXR modulates target genes involved in the formation of hepatic fatty acids, including sterol regulatory element-binding protein 1c (SREBP-1c), stearoyl-CoA desaturase-1 (SCD-1), and fatty acid synthase (FAS). FA uptake into liver is mediated by CD36 transporters which are regulated by PXR activation. A biologically-based quantitative model for hepatic lipid homeostasis was developed incorporating both mechanisms. Furthermore, the impact of chemical exposure on fatty acid hepatic content via LXR, and/or PXR binding was quantitively investigated by incorporating liver tissue dosimetry, using a physiologically-based pharmacokinetic (PBPK) model, with the hepatic lipid homeostasis model. The overall quantitative model was tested against three known agonists of LXR and/or PXR: T0901317 (both receptors), GW3965 (LXR only), and Rifampicin (PXR only). In agreement with literature, model predictions showed that T0901317 caused severe fatty acid build-up in the liver, with slightly less accumulation observed with Rifampicin exposure, and GW3965 caused little lipid build-up. These results highlight the importance of PXR activation while suggesting that FA *de novo* synthesis alone is not enough to cause appreciable accumulation of lipids in the liver. The chemical exposure-hepatic steatosis overall quantitative model can be used to screen chemicals for their potential to cause hepatic steatosis in view of their exposure levels and toxicological potency obtained from high throughput data for LXR and PXR binding. *This abstract does not necessarily reflect US EPA policy.*

2953 NRF2 Negatively Regulates Primary Ciliation and Hedgehog Signaling

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Primary cilia are lost during cancer development, but the mechanism regulating cilia degeneration is not determined. NRF2 normally protects cells from oxidative, proteotoxic, and metabolic stress, with hyperactivation of NRF2 now considered oncogenic. However, the detailed molecular mechanisms by which uncontrolled NRF2 activation promotes cancer progression remain unclear. We report here that NRF2 suppresses hedgehog (Hh) signaling through Patched 1 (PTCH1), and primary ciliation via p62/SQSTM1. PTCH1, a negative regulator of Hh signaling, is an NRF2-target gene, and as such hyperactivation of NRF2 impairs Hh signaling. NRF2 also suppresses primary cilia formation through p62-dependent inclusion body formation and blockage of BBS4 entrance into cilia. Simultaneous ablation of PTCH1 and p62 completely abolishes NRF2-mediated inhibition of both primary ciliation and Hh signaling. Our findings not only uncover a mechanism by which NRF2 hyperactivation promotes tumor progression via primary cilia degeneration and aberrant Hh signaling, but also reveal a previously unidentified role of NRF2 in controlling a cellular organelle, the primary cilium, and its associated Hh signaling pathway. A better understanding of the crosstalk between NRF2 and primary cilia/Hh signaling could not only open new avenues of cancer therapeutic discovery, but could also have significant implications regarding pathologies other than cancer, including developmental disorders, where improper primary ciliation and Hh signaling play a major role. *Supported by the following NIH grants: R01 DK109555, R01 ES026845, and P42 ES004940 to D.D.Z., as well as P30 ES006694.*

2954 Estimation of Toluene Exposure in Air from BMA (S-Benzylmercapturic Acid) Urinary Measures Using a Reverse Dosimetry Approach Based on Physiologically Based Pharmacokinetic Modeling

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Biomonitoring is now an essential component of national health surveys to assess human exposure to chemical contaminants. In exposure assessment, there is evidence that the direct reconstruction of population external exposure from biological measurements (generally on parent compounds) can be done by a reverse dosimetry approach using physiologically based pharmacokinetic (PBPK) models, and steady state assumptions. The objectives of this work are to estimate individual toluene exposure in air from urinary BMA (S-benzylmercapturic acid) measurements collected in six adult Canadians by Health Canada, and to evaluate the contribution of the variability in urinary spot-sample measurements on the overall uncertainty related to toluene exposure estimates based on reverse dosimetry PBPK modeling approaches compared to the use of single 24-h urines measurements. To do so, both exposure assessment techniques were developed for each individual, namely the estimate of toluene exposure in air from a concentration of BMA measured on 24-h urines (24-h-BMA) and from a distribution of daily urinary BMA spot measurements (ds-BMA). Individual distributions of physiological parameters are described based on age, weight, size and sex. Monte Carlo simulations with PBPK models were used to generate Exposure Conversion Factor tables to convert ds-BMA (and 24-h-BMA) into toluene air levels. Based on the approach relying on ds-BMA, the ratio between the 95% probability of predicted toluene concentration and its 50% probability in the individuals studied varied between 1.2 and 1.4, while that based on 24-h-BMA varied between 1 and 1.1 (rounded values). These results point out that computing estimates of toluene air concentrations based on 24-h-BMA generates a lower level of uncertainty. They also suggest an average relative contribution of intra-individual variability in spot measurements to the overall uncertainty based on ds-BMA of about 20%. Toluene levels estimated herein (0.0078-0.0138 ppm) are similar to that previously estimated from Canadian blood measurements and well below Health Canada's maximum recommended chronic air guidelines. Concluding, PBPK modeling and reverse dosimetry can be combined to help interpret biomonitoring data on urinary metabolites of environmental volatile chemicals and assess the magnitude of the related uncertainty.

2955 Development of a Virtual Population Model of Thyroid Hormone Regulation for Safety Assessment of Endocrine Disrupting Chemicals

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Inferring human health risk of environmental exposures to endocrine disrupting chemicals (EDCs) from *in vitro* testing data is a challenging task. It requires consideration of the uncertainties and variabilities inherent to the *in vitro* to *in vivo* extrapolation (IVIVE) process and those in the human endocrine systems. To this end, a dynamic model explicitly capturing the inter-individual variability of a human population will be useful. In this study we aim to establish such a population model of the thyroid hormone system by utilizing the thyroid profile dataset in the National Health and Nutrition Examination Survey (NHANES) from 2007-2012. After eliminating individuals with thyroid cancers and on thyroid medications, we applied a rejection sampling algorithm to obtain a non-stratified sample population based on readjusted sample weights of individuals. The obtained sample population contains measured plasma levels of free T4, free T3, total T4, total T3, and TSH for over four thousand individuals; it was then used as the input to a multivariate kernel density function to estimate a continuous probability density function for a correlated distribution of these hormones. The resulting density function was then utilized to generate virtual human populations of thyroid hormone pro-

files. A dynamic hypothalamic-pituitary-thyroid (HPT) axis model describing the feedback regulation and free and total T4/T3 kinetics was then subject to parameter estimation by using the virtual human population generated. The parameter estimation process is under several mechanistic constraints imposed by the physiology of the HPT axis. To deal with the issue of parameter correlation, a modified Bayesian-like approach was used that iterates through multiple prior distributions for each parameter to obtain posterior distributions. The resulting parameter distributions are sampled to generate a virtual population model that can be perturbed *in silico* by single and mixture of thyroid EDCs. The model can take *in vitro* thyroid toxicity testing data to predict hormone alterations for a human population, bridging the IVIVE gap. The approach developed here can also generate a virtual subpopulation from the NHANES dataset, such as women at reproductive age that are susceptible to thyroid disruptions, for safety assessment.

2956 Probabilistic Physiologically Based Pharmacokinetic Model for Per- and Polyfluoroalkyl Substances (PFAS) in Beef Cattle and Dairy Cows for Food Safety Assessment

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Perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexanesulfonic acid (PFHxS) are chemicals of major concern among the family of per- and polyfluoroalkyl substances (PFAS) due to their environmental persistence and long half-lives in humans. Dietary intake of these chemicals from milk and edible tissues is considered to be the predominant route for human exposure and thus poses potential health risks. There is no available physiologically based pharmacokinetic (PBPK) model to describe the transfer and distribution of PFAS to milk and edible tissues from contaminated feed in farm animals. This study aimed to develop probabilistic PBPK models for PFOA, PFOS, and PFHxS in beef cattle and dairy cows. Based upon previously published PBPK models for PFOA and PFOS in rodents and humans, this study developed a PBPK model for lactating cows fed with naturally contaminated PFAS-contained hays and silages as well as for beef cattle following a single oral dose of PFOS and PFOA. The PBPK structure for dairy cows contained six compartments: plasma, liver, kidneys, udder, muscle, and rest of body. Preliminary results showed that the model adequately simulated available kinetic data of PFOS in plasma and milk with an estimated coefficient of determination (R^2) over 0.95. Monte Carlo simulation method is being incorporated into the model in order to estimate meat withdrawal interval and milk discard time. Extrapolation of this model to PFOA and PFHxS is ongoing. This model provides a useful tool for safety assessment of beef and milk from cattle exposed to PFOS, and serves as a foundation for extrapolation to other PFAS chemicals to improve food safety assessment.

2957 Development of Additional Workflows for Risk Assessment and Prioritization for the PLETHEM Package

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Over the past three years we have been developing the population life-course exposure to health effects model (PLETHEM) - the open source R package that is designed to provide tools that can be used to bridge the source-to-outcome continuum. Pharmacokinetic modeling is becoming increasingly important in chemical safety decision making, with applications in prioritizing risk assessment by establishing margins of exposure for chemicals and extrapolating hazard across different ages, population cohorts, or occupational classes. As a part of our effort to provide the community with an easy-to-use yet capable modeling tool, we have continued to develop the PLETHEM package. We have created workflows for automated reverse dosimetry and route to route extrapolation, modeling for ecotoxicology applications, and kinetically derived maximum tolerated dose estimation. The automated reverse dosimetry module implements the Discretized Bayesian Approach as described by Tan et al. (2007) along with an easy-to-use interface to run the workflow. Simulation results from existing PBPK models or from models parameterized within PLETHEM can be imported for dose reconstruction. We have also added the ability to perform automated route-to-route extrapolations within PLETHEM. The user can parameterize the model for a given route of exposure using the existing forward dosimetry interface within PLETHEM and use this parameterized model to estimate exposure along a different route using this workflow. Next, as a part of this update, we have added a fish PBPK model described by Peyret et al. (2009), physiological datasets for this model and an easy to use modeling interface to help stakeholders address ecotoxicology concerns. Lastly, we are incorporating into PLETHEM additional features to make kinetically derived maximum tolerated dose (KMD) modeling more accessible. KMD makes use of kinetic modeling to identify dose ranges that avoid saturating ADME processes, which would convolute the interpretation of the study. McFadden et al. (2012) described a statistical method to determine KMD values for toxicokinetic studies. We are adding to PLETHEM an interface that allows users to import their own toxicokinetic datasets or simulated PBPK models from PLETHEM to estimate a KMD. PLETHEM is freely available via the Comprehensive R Archive Network (CRAN). We are also hosting some of these workflows as standalone apps online to provide users with easy access to them.

2959 Combining Evidence to Assess Mutagenicity of Impurities by Supplementing QSAR, Rule-Based, and Read-Across Predictions with Expert Opinions

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In silico toxicity predictions have been established as alternative methods for evaluating the genotoxic potential of chemical impurities or metabolites in safety assessment of pharmaceuticals (ICH M7) or agrochemicals (EFSA). Quantitative structure-activity relationships (QSARs) and expert knowledge-based rules are commonly employed for *in silico* models and are well-suited for DNA-reactive mutagenicity where modes of chemical reactivity are well characterized. While much effort has been dedicated to the *in silico* modelling of one particular endpoint, bacterial reverse mutagenesis, there remains a need to establish a systematic method to consider and incorporate other relevant pieces of evidence including, for example, experimental results from mechanistic analogs to improve confidence in predictions, or the need to evaluate such experimental data with further knowledge in metabolism or reactivity. This study was performed to demonstrate the full workflow necessary in this process using pharmaceutical impurities as examples. Experimental Ames assay results, QSAR and Rule-based predictions, and expert's rationales are lined up in an assessment table, and these data are then combined for the final outcome along with uncertainty estimation. Three studies were included for cases when QSAR and Rule-based results 1) are all in agreement; 2) show equivocal results (e.g., 1,2-diformylhydrazine); 3) are in conflict (e.g., 1,2-diphenylhydrazine). Expert reviews were also included for each case to decide whether to overrule the predictions or recommend the weight-of-evidence final conclusion. This approach pushes the envelope of *in silico* methods to be combined with expert opinions in a weight of evidence mode to achieve reproducible and robust assessments.

2960 Calculated Solvation Energies Predict the Elimination Half-Lives of Perfluoroalkyl Substances in Mammals

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Some perfluoroalkyl substances are highly stable and bioaccumulative contaminants in mammals. The elimination half-lives for some analogs in humans measures in years, not hours. However, the half-lives can vary dramatically based on the analog and species in question. In general, longer-chain perfluoroalkyl analogs have lengthened half-lives in larger mammals. This complicates risk assessment because the points of departure are often serum levels in rodents that cause a health effect. The human equivalent dose (HED) that would result in an equivalent serum level is calculated using estimates for half-life, volume of distribution, and absorbed fraction. Because the half-life may differ drastically between rodents and humans, it has a large effect on the final HED. Relatively few perfluoroalkyls have elimination half-lives measured in multiple species. This data gap is exacerbated by increasing use of shorter-chain analogs that are less persistent and therefore perceived to be less toxic. Because most perfluoroalkyls are not modified metabolically, we hypothesized that the half-life is driven by the physical properties of the substances. However, standard prediction tools (EPIsuite, ACD/Labs) for these properties may lack parameterization for their unique chemistry. Therefore, *ab initio* quantum mechanical calculations with the Poisson-Boltzmann solvation model were performed on perfluoroalkyls with experimental half-lives. The resulting solvation energies for water and cyclohexane are able to explain more than 80% of the variance in experimental half-lives in a simple multiple least squares regression model that includes human, monkey, mouse, and rat species. The model can be viewed as a first step toward establishing elimination half-lives based on a structure activity relationship for perfluoroalkyls and may allow for a rough estimate of the bioaccumulative potential of alter-

native perfluoroalkyls. *Disclaimer: the findings and conclusions in this presentation have not been formally disseminated by [the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry and should not be construed to represent any agency determination or policy.*

2961 Drug-Induced Liver Injury Severity and Toxicity (DILIst): Binary Classification of 1303 Drugs by Human Hepatotoxicity

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Drug-induced liver injury (DILI) is one of the primary challenges for drug development in part because of the limited success with existing preclinical models. This concern has led to significant efforts in evaluating alternative methods, particularly animal-free approaches, for predicting DILI in humans. Most of these methods requires a large drug list with known DILI severity and toxicity that distinguish DILI positives from negatives. Previously, we reported DILIrank that consisted of 775 drugs and their DILI classifications, assessed using FDA approved drug labeling information. In this study, we augmented DILIrank with four large literature datasets (N>350 drugs) using a statistical approach to generate DILIst (DILI severity and toxicity). The augmentation was carried out separately for each DILI class (i.e., positive or negative) and resulted in a DILIst database of 1303 drugs of which 789 were DILI positives (increased 69% from DILIrank) and 514 were DILI negatives (increased by 66%). DILIst is an invaluable resource for the community to improve DILI research in the areas of elucidation of mechanisms, predictive model development and biomarker identification, and provides additional opportunities to exploit the potential of emerging technologies. *Disclaimer: The views presented in this paper do not necessarily reflect current or future opinion or policy of the US FDA. Any mention of commercial products is for clarification and not intended as endorsement.*

2962 3D-SDAR Classification Models for Large and Diverse Sets of Opioid Receptors Binders: Structural Factors Affecting Binding to Mu, Kappa, and Delta Opioid Receptors

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Addiction is a complex behavioral phenomenon in which naturally occurring or synthetic chemicals through their binding to a variety of neuroreceptors, modulate the response of the reward system, resulting in compulsive substance-seeking (Can. Med. Assoc. J. 164.6, 2001, 817). Among these, the opioid receptor family (OR) plays a critical role in the addiction to powerful prescription and illicit drugs. As more than 700 new psychoactive substances were illegally sold between 2009 and 2016, most of them lacking basic toxicological and pharmacological profiles, molecular modeling approaches that could quickly and reliably fill in the gaps in our knowledge are highly desirable. A grid-based, alignment independent technique utilizing fingerprints constructed from NMR chemical shifts and interatomic distances (named 3D-SDAR) was used for model building, validation and interpretation. To generate balanced modeling and "blind" prediction subsets, all chemicals from the initial sets of 3594, 2942 and 2420 MOR, KOR and DOR binders were sorted according to their molecular weight and every 3rd compound from these three lists was moved out to form the prediction subsets. The remaining 2/3 of the chemicals constituted the modeling subsets which were then split repeatedly into training and hold-out test set pairs used for model building and internal validation. After 100 randomizations the aggregated predicted values for the training, hold-out and prediction subsets were averaged individually for each compound and each subset and a cut-off value of 0.5 was used to classify the chemicals as either binders (>0.5) or non-binders (≤0.5). The accuracy, sensitivity and specificity for the "blind" prediction subsets consistently exceeded 0.8 and were significantly higher after the removal of the inconclusive predictions near the cut-off. The AUC values for all prediction subsets exceeded 0.88. 3D-SDAR mapping identified the morphinan and benzomorphan backbones as universal and non-selective components of OR binders, whereas specific functional groups such as 3,4-dimethyl-4-(3-hydroxyphenyl) piperidine, 3-pyrrolidinol and benzamide were found to result in substances that are selective to MOR, KOR and DOR, respectively. Furthermore, a cyclopropylmethyl moiety linked to the basic nitrogen atom of both morphinan and benzomorphan derivatives was found to enhance the affinity towards all OR subtypes.

2963 Improving the Read-Across Assessment Framework (RAAF) for Better Assessing Read-Across Predictions

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From the viewpoint of animal welfare, alternative methods for the safety assessment of chemicals is becoming more vital. Among the alternative methods, read-across is importantly used to fulfil the requirement for information under regulatory frameworks, such as the registration, evaluation, authorization and restriction of chemicals (REACH) regulation. As a systematic framework to assess the read-across cases under REACH, the Read-Across Assessment Framework (RAAF) was developed by the European Chemicals Agency (ECHA). The validity and reliability of the read-across is judged according to the Assessment Elements (AEs) in the RAAF. Basically, the RAAF is not created for a specific endpoint and its AEs are scientific aspects commonly required for all read-across cases (e.g. data quality, category boundary, underlying mechanisms, etc.). However, for the read-across of a specific endpoint, more specific AEs would be more useful for users to assess their read-across predictions. Therefore, in this study, we tried to improve the original RAAF for more practical applications and developed a RAAF for skin corrosion/irritation read-across (RAAF-SCI) by setting more specific assessment procedures based on the original AEs. For example, category boundaries were determined on the basis of octanol-water partition coefficient, melting point, boiling point and molecular weight, the common underlying mechanisms were evaluated based on the profiling results by the OECD QSAR Toolbox, and source data quality was assessed by referring to the publically available reliability scores. In order to evaluate the effectiveness of our improved RAAF-SCI, we applied it to the read-across predictions of 4 chemicals whose skin irritation data already existed. Initially the category of the target chemical was created by the predefined procedure using the OECD QSAR Toolbox [SAR QSAR Environ Res, v30, 2019 p279]. The category was then assessed by the RAAF-SCI and improved if necessary. For example, if the target chemical was outside the boundary of the category, analogues were added so that the target chemical fell into the category. Finally, the read-across from the improved category members could reproduce the skin irritation potential of the target chemicals. As a result, our RAAF-SCI with improved AEs gave more reliable read-across predictions.

2964 Quantitative Systems Toxicology (QST) Reproduces Species Differences in PF-04895162 Liver Safety Due to Combined Mitochondrial and Bile Acid Toxicity

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PF-04895162 (ICA-105665), a drug in development for the treatment of epilepsy, was terminated after transaminase elevations (up to grade 3) were observed in healthy volunteers (NCT01691274). Human hepatotoxicity was unexpected, because liver safety concerns had not been raised in preclinical safety studies (Aleo et al. 2019). The purpose of our investigation was to better understand the mechanisms underlying the apparent species differences, between rat and human, in liver safety. We retrospectively analyzed PF-04895162 using a computational representation of drug induced liver injury, DILIsym, which integrates *in vitro* data of hepatotoxic mechanisms with *in vivo* predictions of liver exposure to mechanistically simulate hepatotoxicity. The *in vitro* data include drug inhibition of bile acid (BA) transporters (human BSEP, NTCP, MRP3 and MRP4, as well as, rat Bsep, Mrp3 and Ntcp) and drug-induced mitochondrial dysfunction (using the Seahorse XF Analyzer). PF-04895162 weakly interacted with BA transporters (IC_{50} values >100μM) and mildly reduced mitochondrial function. Physiologically based pharmacokinetic (PBPK) models were fit to pre-clinical and clinical plasma concentrations and used to predict *in vivo* liver concentrations. DILIsym integrated the *in vitro* data and PBPK models to predict hepatotoxicity in both species. Simulations were conducted in SimPops, which represent population variability, including pathways that regulate sensitivity to certain hepatotoxic mechanisms. The simulations reproduced the observed human hepatotoxicity and the lack of rat hepatotoxicity. Simulated hepatotoxicity was multifactorial. Simulations showed higher liver exposure in humans than in rats. Elimination of drug effects on either mechanism wholly abrogated simulated injury; thus, both mechanisms were required. The interaction of two hepatotoxic mechanisms explains how the mild effects observed *in vitro* could plausibly translate to hepatotoxicity *in vivo*. Importantly, the mechanistic interaction is not readily foreseeable given the *in vitro* data alone. This study reproduces species differences using a QST approach and supports the contention that QST tools have the potential to identify latent hepatotoxic risks.

2965 Application of the CiPA *In Silico* Model in Early Drug Research: Validation Results for Different Drug Development Phases

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The CiPA (comprehensive *in vitro* Proarrhythmia Assay) initiative proposes a novel screening paradigm for the assessment of proarrhythmic liabilities. The core element is an *in silico* cellular simulation model (O'Hara/Rudy type) combining ion channel *in vitro* data to reconstruct the cardiac action potential. The CiPA paradigm is intended to refine the ICH S7B/E14 guideline and the clinical thorough QT (TQT) study in development. This study investigated if the CiPA model is also applicable in the research drug optimization phase to early eliminate drug candidates with proarrhythmic risk before entering development. We first evaluated the 28 drugs (9 low, 11 intermediate, 8 high risk) that were also used to validate the CiPA model. Various simplification steps were applied to simulate the early research phase when limited data sets are available for decisions. So we predicted the proarrhythmic risk by reducing the number of originally 7 down to the 3 main ion channels hERG, Cav1.2 and Nav1.5 (peak) typically screened in early research. Then we excluded the dynamic binding parameters and just used IC50 values for simulation. With these settings, the correct classification of almost all high and low risk drugs of the 28 CiPA compounds could be confirmed using qNet values at 4x Cmax. However, both false negatives and false positives were observed. The CiPA model was also applied to internal drug candidates and proarrhythmic risk was validated against results from the Purkinje fiber assay as an important follow up assay in early research. Finally, since many drugs in early optimization are first theoretically designed, the IC50 values of hERG, Cav1.2 and Nav1.5 were also predicted based on their chemical structure using in-house QSAR models. So when using predicted IC50 values for the CiPA simulation, depending on the QSAR model's applicability domain and chemical series, most of the positives were correctly predicted with sensitivities up to 80-90% (average prediction accuracy was 70%). The accuracy could be increased when predicted IC50 values were replaced by measured data. These validation results show that a combination of the CiPA model using predicted or measured ion channel data and confirmation by the Purkinje fiber assay can successfully be applied in R&D to rank and early eliminate drug candidates with proarrhythmic liabilities but cannot be currently used for quantitative safety margin evaluation.

2966 Data-Driven Selection of Biologically Diverse Cell Lines for Chemical Bioactivity Screening Using Content Maximization

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Many research organizations are exploring the use of high-throughput profiling (HTP) assays for *in vitro* hazard evaluation. HTP assays can be used to characterize the response of many human-derived cell types to chemicals. However, no single cell type will express all the potential molecular targets and pathways that are present in the human body. Therefore, an envisioned approach for HTP assays in bioactivity screening involves panels of cells that express complimentary (i.e. partially non-overlapping) sets of molecular targets to address a broad swath of human biological diversity. Here, we present a data-driven approach for selection of biologically diverse cell lines for HTP screening based on basal gene expression. First, the cancer cell line encyclopedia (CCLE) Affymetrix transcriptomics dataset was RMA-normalized and a pair-wise Euclidean distance matrix was calculated. Three cell lines of programmatic interest (MCF-7, U-2 OS, HepG2) were selected as anchors (i.e. initial vertices) and ten more biologically diverse cell lines were selected by identifying the group of cells within CCLE that maximized the content (i.e. Cayley-Menger determinant) of a multidimensional simplex. This approach identified cell lines with a variety of tissue origins (immune, fibroblast, lung, upper aerodigestive tract, skin, CNS and kidney) that were non-redundant with the tissue origins of the anchor lines (breast, bone, liver). These cell lines, along with a set of thirteen hTERT-immortalized primary cell lines from various tissues and 2-D HepaRG cells were profiled using whole transcriptome TempO-Seq. The data was used to map the diversity of molecular targets and pathways expressed across the 30 total cell lines. Cell lines from similar tissues tended to have more similar gene expression profiles than cell lines from disparate tissues. The hTERT cell lines had less diversity in gene expression than the cancer cell lines. The content maximization algorithm was applied to the TempO-Seq data to generate ranked lists of cell lines that progressively increases the amount of biological diversity given a particular set of anchor cell lines. This data-driven method can be used in an unbiased manner for populating cell line panels for HTP-based toxicity screening. *This abstract does not reflect US EPA policy.*

2967 Comparison of CATMoS Acute Oral Toxicity GHS Classifications with Historical *In Silico* Estimates and *In Vivo* Test Data

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Computational toxicology has rapidly progressed in capability in the last decade as alternative methods have become more widely available to attempt to reduce reliance on animal testing while providing equivalent or more accurate information for human health risk assessments. As part of this effort, significant resources have been dedicated to developing comprehensive tools to aid those conducting *in silico* assessments, including the development of the Open Structure-Activity/Property Relationship App (OPERA) by the National Toxicology Program (NTP). To evaluate the performance of the OPERA Collaborative Acute Toxicity Modeling Suite (CATMoS) Acute Lethality Consensus model, the model's predictions were compared to historical acute oral lethality computational assessments conducted by a 3M weight of evidence (WoE) approach (n=81) that included analysis of (Q)SAR predictions and read-across from structural analogs. When comparing the results, the CATMos predicted GHS class agreed with the 3M WoE prediction for 78% of the substances (63/81). In instances where there was disagreement (n=18), the 3M WoE prediction was more conservative (a lower GHS class was predicted) for 17/18 of those substances. Additionally, the CATMoS model's performance was compared to a set of proprietary 3M chemistries with experimental *in vivo* oral LD_{50} values and corresponding GHS classifications (n=70). When testing CATMoS against the experimental test set, the model correctly predicted the GHS classification for 77% of the substances (54/70). For substances that were misclassified by the model (n=16), 7 of the predictions were more conservative (a lower GHS class was predicted) compared to the experimental data. Substances where the lethality was under-predicted most often contained metals or double or triple-bonded nitrogen. Overall, the CATMoS Acute Lethality consensus model performed well when tested against a diverse test set that included hydrocarbons, fluorocarbons, and structures will a wide variety of phosphorous, sulfur and some nitrogen-containing functional groups that can often pose challenges for statistical models that rely on a single prediction rather than a consensus approach. However, the consensus model was less conservative in some instances compared to a WoE approach that considered (Q)SAR predictions and read-across from structural analogs. Further evaluations will include attempts to identify the types of chemical classes or functional groups that CATMoS was not able to correctly classify.

2968 ToxSIBAR: Predicting Toxicity with Similarity-Based Descriptors

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The concept of similarity is broadly implemented in predictive toxicology, assuming that chemical compounds with similar chemical structure, similar pharmacological target profiles, similar metabolic, toxicodynamic and or toxicokinetic profiles will exert similar toxicological effects. Being widely known as read-across approaches, such concepts can aid as alternative tools to animal testing for hazard assessment. In addition to a concept aiming to group (or position) compounds based on some sort of similarity measure, compounds with known toxicity can also be used to build a predictive computational model in order to forecast the toxicity of a compound with unknown adverse effect. A prerequisite however, is the availability of enough chemical data as well as the employment of relevant features (describing/encrypting the chemical compounds) being able to sufficiently capture the essentials of that toxic event. The latter, is one of the biggest challenges in computational toxicology nowadays since mechanistic information of the toxic event under study can seldomly sufficiently be encrypted in the chemical structures and derived descriptors. We are proposing a new methodology, utilizing the concept of read-across in the framework of a predictive modeling approach. The ToxSIBAR concept is based on previous work where (euclidean) distances of the training set compounds to a set of reference compounds were utilized as input features for building QSAR models for predicting e.g., transport inhibition. We applied this concept to an *in vivo* dataset of >1000 diverse compounds measured as positive or negative for hepatic steatosis in rodent studies with repeated oral exposure by using different reference compound sets (e.g., randomly chosen; based on maximum diversity) and by applying different basic descriptors and classifiers. Interestingly, the models utilizing the ToxSIBAR descriptors as input outperformed the baseline models highlighting the potential of the method to be developed and optimized further.

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2969 Quantitative Systems Toxicology Modeling of Cisplatin Nephrotoxicity Using *In Vitro* Assays of Proximal Tubule Epithelial Cells for Mechanistic Toxicity Pathways

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Cisplatin-induced nephrotoxicity results in acute kidney injury (AKI) and is caused by various cellular mechanisms, including mitochondrial dysfunction, oxidative stress, and others. AKI mechanisms of cisplatin and several other nephrotoxic drugs remain incompletely understood. Quantitative system toxicology (QST) offers promise for better understanding of drug induced AKI through mechanistic representation of the underlying toxicity pathways. We developed a QST model of cisplatin induced AKI using *in vitro* assay data to characterize injury pathways. We employed RENAsym, a QST model of drug-induced AKI, to quantify cellular toxicity and injury mechanisms of nephrotoxicants. RENAsym represents aspects of renal proximal tubule epithelial cells (RPTEC) including cell life cycle, bioenergetics, drug-induced cell death pathways, and biomarker (αGST) responses. For mechanistic representation of cisplatin induced AKI, we analyzed data from a 2D *in vitro* system (collected by Cyprotex, Inc.) of RPTEC. Seahorse XF analyzer and high content imaging (HCI) were used to quantify cisplatin-induced mitochondrial dysfunction and oxidative stress. Seahorse mitochondrial dysfunction assays show a substantial decline in oxygen consumption rate at 24 hours, suggesting dose-dependent ETC inhibition by cisplatin. Similarly, HCI data depicts a dose-dependent rise in oxidative stress, both at 24 hours and 9 days. Simulations using RENAsym predict dose-dependent toxicity as quantified by elevations in αGST, a biomarker that marks RPTEC death. A simulated single high dose of 533 mg/m^2 i.v. cisplatin results in 14 fold change in αGST, while a simulated clinical dose of 100 mg/m^2 shows 1.4. This result is in qualitative agreement with 3.4 fold change observed in a clinical study where patients administered 100 mg/m^2 i.v. cisplatin exhibited 20% incidence of AKI [1]. RENAsym simulations predicted dose-dependent AKI in human exposed to cisplatin, in qualitative agreement with clinical data. RENAsym shows promise in combining QST modeling and *in vitro* assay data to provide a unique tool for drug-induced AKI prediction. *Funding: NIDD SBIR grant [1] Ummer et al. International journal of Bioscience, Biochemistry and Bioinformatics, Vol. 2, No. 4, July 2012.*

2970 Updated Dermal Sensitization Thresholds Derived Using an *In Silico* Expert System and an Expanded Local Lymph Node Assay Dataset

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When toxicologists need to conduct a Quantitative Risk Assessment (QRA) of a potential skin sensitiser lacking *in vivo* toxicity data, one available approach is the Dermal Sensitisation Threshold (DST). The DST acts as a surrogate point of departure within the QRA when exposure to the chemical is expected to be low, representing a worst-case scenario based on the known skin sensitisation potency of hundreds of chemicals. Two values have previously been published: a non-reactive DST of 900 µg/cm^2 based on Local Lymph Node Assay (LLNA) data for 38 sensitisers judged to be non-reactive by human experts, and a reactive DST of 64 µg/cm^2 based on LLNA data for 233 reactive sensitisers. This study sought to update these DST values using an expanded LLNA dataset, and to investigate whether the chemical reactivity could be assigned *in silico*. An expanded LLNA dataset containing 1,175 chemicals was collected from the public domain and curated in-house. The potency values in this dataset fitted a similar gamma distribution to the original dataset. The skin sensitisation structural alerts within Derek Nexus, an *in silico* expert system for predicting toxicity, were used to assign each chemical as reactive or non-reactive. The 454 reactive sensitisers resulted in a reactive DST of 59 µg/cm^2, whilst the 109 non-reactive sensitisers led to a preliminary non-reactive DST of 290 µg/cm^2. The updated reactive DST (59 µg/cm^2) was remarkably similar to the original value (64 µg/cm^2) despite being based on almost twice as many chemicals, indicating the robustness of the reactive DST. However, the updated non-reactive DST (290 µg/cm^2), based on almost 3 times as many chemicals, was approximately 3 times smaller than the original value (900 µg/cm^2). A review of the 109 non-reactive sensitisers highlighted chemicals requiring autoxidation, questionable dose-response data and possible false positive irritant responses. As an example of the impact these data points can have, removal of the LLNA data for 3 salicylate esters (which are likely to be irritants rather than sensitisers) would cause the non-reactive DST to increase to 320 µg/cm^2.

2971 Developing Semantic Technology for Toxicology Applications: Interdisciplinary Collaborations and Community Development

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Modern toxicology is evolving to leverage data science methodologies to better address complex public health concerns. Understanding the health impacts of environmental exposures requires working across a variety of domains and data types that are often siloed and require manual curation and extraction. Ontologies and semantic engineering can facilitate meaningful data integration, but existing semantic standards have not been widely used in toxicology. Development of semantic standards for toxicology requires sustained interdisciplinary collaborations. One place where the community came together to discuss this need is the "Computable Exposures" workshop, held at Oregon State University in September 2019. Ontologists, toxicologists, epidemiologists, exposure scientists, ecotoxicologists, clinicians, computer scientists, computational biologists, and data scientists from academia, government, and industry were in attendance. Here we describe community-building efforts, standards development, and plans for future work. Objectives include building a semantic exposure data model using the Environmental Conditions, Treatments, and Exposures Ontology (ECTO), developing toxicology-driven use cases and competency questions, creating a mailing list, and planning a larger computable exposures conference. Fifteen use cases were developed during the workshop, including the use of semantic technology to complete Adverse Outcomes Pathway (AOP) and Aggregate Exposure Pathway (AEP) given the initiating and terminal key events. Broader adoption of ontologies, together with increased data sharing, has the potential to improve a toxicologist's ability to integrate, navigate, and analyze vast amounts of heterogeneous data—allowing for more rapid safety assessment of chemical and environmental exposures, and increased understanding of underlying biological mechanisms.

2972 Toward Mechanistic-Based Assessment: Rapid Elucidation of the Key Molecular Pathways Underlying Diketone-Induced Bronchiolitis Obliterans Using Phenotypic and Transcriptomic Profiling

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New approach methods based on mechanistic reasoning have the potential to provide more human-relevant information for chemical safety assessment. In particular, genome-wide transcriptomics may be used to rapidly elucidate the molecular pathways underpinning the key events of an adverse outcome. Recent advances in transcriptomic technology have enabled more cost-effective and higher throughput assays. However, the relevancy of the identified molecular pathways is still largely limited by the biological models used, and their specificity to an adverse outcome. We have developed a platform called Toxicity Mode of Action Discovery (ToxMAD) Platform to overcome these challenges. To demonstrate the application of ToxMAD, we performed a case study on diketone-induced bronchiolitis obliterans (BO). Diketones are flavoring additives shown to be associated with BO in popcorn factory workers. *Firstly,* we performed a literature review to identify the key events associated with the progression of BO under *in vivo* diketone exposures. *Secondly,* we identified *in vitro* human cell models sensitive to a chemical of interest using high-throughput imaging-based phenotypic profiling (HIPPTox). Among the three tested human cell models, a lung bronchial epithelial cell line, BEAS-2B, was found to be the most sensitive to *diketones,* including diacetyl and 2,3-pentanedione. Thirdly, we used high-throughput transcriptomics (HTTr) to identify differentially expressed genes (DEGs) induced by the two chemicals, and 849 and 533 DEGs (Adj P-value<0.05) were found to be induced by them, respectively. Interestingly, the gene expression profiles induced by these two diketones are positively correlated (r=0.929, p=2.2x10^{-16}) suggesting the same modes of action were induced. Furthermore, many of the DEGs

were also found in a previous rodent transcriptomics study of 2,3-pentanedione. Finally, we developed bioinformatic methods to identify key pathways and their corresponding DEGs that are relevant to the key events of BO. These genes may be used as markers to compare the potency and evaluate the potential hazards of other diketones or related compounds that may cause BO.

2973 Predicting Subchronic and Chronic Animal Toxicity from *In Vitro* High Content Imaging Data Using PBTK and Machine Learning

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A major challenge in toxicity testing in the 21st century is to predict animal toxicity from *in vitro* studies. Here, we utilized physiologically based toxicokinetic (PBTK) modeling and machine learning (ML) to predict mouse, rat, and dog liver toxicity from high content imaging (HCI) data obtained by measuring HepG2 cell responses to 967 chemical treatments across 10 endpoints and 3 time points (1h, 24h, and 72h). First, the HCI data were normalized to generate z-score data for p53, c-Jun, H2A.X, PH3, α-tubulin, mitochondrial membrane potential, mitochondrial mass, cell cycle arrest, nuclear size, and cell number. Second, lowest-observed adverse effect level (LOAEL) values for chemicals from subchronic/chronic studies in mouse (75/154), rat (161/160), and dog (69/113) were obtained from the ToxRef database. Third, LOAEL values were converted to average venous concentrations using PBTK to match the *in vitro* treatment protocol. Fourth, each *in vitro* treatment was associated with a toxicity class as follows: nontoxic if the venous concentration corresponding to LOAEL was greater than *in vitro* concentration, and toxic otherwise. Finally, 5 ML algorithms (k-nearest neighbors (kNN), Random forest (RF), support vector machine (SVM), decision trees (DT) and naïve Bayes (NB)) were used to evaluate the accuracy for predicting toxicity in each study type and species by each *in vitro* time point. Lastly, we created balanced data using B-splines to interpolate the HCI concentration-response data at untested concentrations. The mean area under the receiver operating characteristic curve (AUC) for chronic and subchronic imbalanced data were 0.7 (SD 0.01) and 0.72 (SD 0.01), respectively. The predictive performance was greater for balanced datasets with a mean AUC of 0.73 (SD 0.01) and 0.79 (SD 0.01) for chronic and subchronic toxicity, respectively. RF was the best algorithm to predict subchronic liver toxicity with AUC 0.96 (SD 0.005) for mice, 0.9 (SD 0.004) for rat and 0.84 (SD 0.01) for dog. For chronic studies, the most accurate classifiers were RF for mice 0.87 (SD 0.01), kNN for rat 0.82 (SD 0.005) and dog 0.79 (SD 0.01). The best prediction for mouse subchronic/chronic liver toxicity was obtained from 1h/24h HepG2 data, whereas for rat and dog, the highest score was obtained at 24h/1h. Our findings suggest the utility of a new approach for linking *in vitro* data to *in vivo* outcomes using machine learning. *This abstract does not reflect EPA policy.*

2974 Toxicants Associated with Spontaneous Abortion in the Comparative Toxicogenomics Database

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Up to 70% of all pregnancies result in either implantation failure or spontaneous abortion (SA). Adverse pregnancy outcomes are particularly challenging to evaluate in any one *in vitro* or *in vivo* model, thus we lack a comprehensive understanding of how chemicals exposures affect the risk of SA. Our goal was to identify chemicals with a high number of interactions with SA genes, based on known toxicogenomic responses. We used reference SA and chemical gene lists from the Comparative Toxicogenomics Database in human, mouse, and rat. We prioritized chemicals (n=25) found in Superfund sites. For chemical-disease gene sets of sufficient size (n=13 chemicals, n=20 comparisons), chi-squared enrichment tests and proportional reporting ratios (PRR) were calculated. Among the SA genes, we assessed enrichment for Gene Ontology defined biological processes. In humans, all chemicals tested were highly enriched for SA gene overlap (all $p<0.001$; parathion PRR=7, cadmium PRR=6.5, lead PRR=3.9, arsenic PRR=3.5, atrazine PRR=2.8). In mice, highest enrichment ($p<0.001$) was observed for naphthalene (PRR=16.1), cadmium (PRR=12.8), arsenic (PRR=11.6), and carbon tetrachloride (PRR=7.7). In rats, we observed highest enrichment ($p<0.001$) for cadmium (PRR=8.7), carbon tetrachloride (PRR=8.3), and dieldrin (PRR=5.3). SA genes were overrepresented in the following biological processes: inflammatory response (q=0.001), collagen metabolic process ($q=1x10^{-13}$), and vascular development (q=0.005). We observed chemical gene sets (parathion, cadmium, naphthalene, carbon tetrachloride, arsenic, lead, dieldrin, and atrazine) were highly enriched for SA genes. Our findings have critical public health implications for successful pregnancies and the interpretation of environmental pregnancy cohort analyses. These findings also have implications for the design of toxicology studies investigating the end point of spontaneous abortion.

2975 Weight of Evidence Assessment of Genotoxicity Based on a Battery of Genetic Toxicity Assays: Improvement of Decision Making for Genotoxic Carcinogens

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Determining whether a compound is mutagenic and/or genotoxic is an important decision point in regulatory risk assessment workflows. In addition to the obvious need to differentiate genotoxic and non-genotoxic modes of carcinogenicity, the early decision steps in the Threshold of Toxicological Concern (TTC) framework require knowledge of genotoxicity, with a separate decision tree for suspected genotoxic carcinogens. Which assays should be used and how many need to be positive before a chemical is termed "genotoxic" are common questions. The issue is complicated by the long-held understanding that genotoxic carcinogens are more potent than those acting by non-genotoxicity mechanisms. Thus, there is a need to define and identify genotoxic carcinogens. Regulatory guidance, e.g. from the US Environmental Protection Agency (EPA), recommends a hierarchical data preference method with a weight of evidence (WoE) approach. As a result, decisions on mutagenicity or genotoxicity are often made by expert opinion utilizing and interpreting multiple streams of data from *in vitro* and *in vivo* assays through WoE. The aim of this study was to define genotoxic carcinogens from publicly available genetic toxicity data applying WoE approaches. From the updated CPDB (Carcinogenicity Potency Database), 549 selected compounds were identified, for which BMDL10 and TD10 calculations were possible to be used for Cancer-TTC analysis. Genetic toxicity data for these putative carcinogens were sourced from diverse sources including NTP, FDA, ECHA, EFSA and the open literature as well as ChemTunes.ToxGPS. The dataset was evaluated to rate study reliability, and the available data were then combined to define whether a compound is a genotoxic carcinogen. Two WoE approaches were compared, the first based on rationales by experts using both experimental, when available, and (quantitative) structure-activity relationship ((Q)SAR) predictions. The second employed training a Bayesian Network to define a latent variable for "genetic toxicity" given a battery of assay data and *in silico* predictions. Validating the identification of genotoxic and non-genotoxic carcinogens was performed utilizing available data from the EFSA and ECHA databases. This approach to systematically define "genotoxicity" empowers and extends the value of (Q)SAR in safety/risk assessment.

2976 NICEATM Computational Tools and Resources Supporting Alternative Test Method Development and Evaluation

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The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) develops and evaluates alternatives to animal use for chemical safety testing. To support these activities, NICEATM has developed a set of computational tools and resources in partnership with federal agencies, industry, and academia. This presentation highlights two resources that make data and computational tools more accessible to our stakeholders: the Open Structure-activity/property Relationship App (OPERA) and the Integrated Chemical Environment (ICE). OPERA is a suite of QSAR models to predict physicochemical and pharmacokinetic properties often needed in modeling. OPERA also includes a set of models built using global collaborative crowdsourcing approaches to construct consensus models for various toxicity endpoints. CERAPP and CoMPARA are consensus models that predict estrogen and androgen receptor pathway activity, respectively. The newest model, CATMoS, is a consensus model providing predictions for acute oral systemic toxicity (LD50) and hazard categories. OPERA is available as a standalone downloadable program with graphical user interface and command line options. ICE is an online database that provides users access to *in vivo*, *in vitro*, and *in silico* data for a range of toxicity endpoints, including curated Tox21 high-throughput screening data for >9,000 chemicals and OPERA predictions for >700,000 chemicals. ICE also includes a growing suite of tools such as a web-based *in vitro* to *in vivo* extrapolation tool allowing users to

compare predicted exposures from *in vitro* bioactivity concentrations to doses from *in vivo* animal studies. For test method developers and evaluators, curated lists of reference chemicals with known effects are available along with their supporting data. Features of both tools and example use cases in the context of chemical evaluation will be presented. *This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.*

2977 Automated Applications of Ontologies to Standardize Developmental Toxicology Study Extractions

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Extraction of toxicological endpoints from primary sources is a central component of systematic reviews in human health risk assessment. For extracted data to be comparable and therefore useful for analyses such as calculating chemical-specific effects, establishing reference datasets for validation of new approaches, and computational modeling purposes, consistent language should be used for endpoint descriptions. However, primary source language describing treatment-related effects can vary greatly, resulting in large labor efforts to manually standardize extractions before data is fit for use. To minimize these labor efforts, we developed Python code that automates application of pre-existing ontologies and controlled vocabularies to extracted endpoints. Our approach was initially developed to apply a subset of Unified Medical Language System (UMLS) codes (i.e., those used previously in ToxRefDB as described in Watford et al. 2019) along with German Federal Institute for Risk Assessment (BfR) DevTox ontology codes and OECD endpoint vocabularies to roughly 36,000 extractions from developmental toxicology studies conducted by the National Toxicology Program. Extractions were recorded based on the original study report language. Our code was able to automatically apply standardized ontologies to 82% of these extractions. The code was then applied to 6,400 extractions from ECHA developmental toxicology studies and was able to automatically standardize 79% of the extractions. Of extractions that were standardized, about 6% required additional manual curation to correct inaccurate matches (e.g., "missing tail" tagged as "double-tipped tail"). This low error rate is due to optimizing the code for precision. Extractions from both datasets that were not standardized tended to be out of scope of developmental toxicology endpoints (e.g., "number of litters"). We estimate that we reduced time spent standardizing the language for these two datasets from 350 to 50 hours, assuming 30 seconds per extraction. We would predict similar results if this approach was applied to other legacy developmental toxicology extraction datasets. In addition, the design of the code ensures that it can be easily expanded beyond developmental toxicology endpoints for application to other study types, which would improve the utility of legacy datasets for use in modeling or other analyses.

2978 Improving Identification of Neuroactive Compounds Using Temporal Information from Microelectrode Array Recordings of Cortical Neural Networks and a Semi-Supervised Classification Algorithm

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Human exposure to environmental chemicals can result in acute neurotoxicity (NT), negatively impacting brain activity. *In vitro* microelectrode array recordings of neural network function following chemical exposure can be used to screen chemicals for NT hazard. These recordings capture temporal (from min to days) and spatial aspects of action potential activity as described by a set of network parameters (NPs). To determine if a compound is neuroactive, global NPs are extracted from 40 min neural recordings resulting in loss of temporal information (TI). The TI could improve identification of compound fingerprints and/or provide information on the mechanisms of action that mediate a neural network response after acute exposure of a compound. Here, data from 384 previously tested compounds were used to explore the properties of the TI to screen for acute neuroactive compounds using the response from a single high concentration (nominally 40 μM) and a window analysis technique. From recordings on day *in vitro* 12, a total of 19 NPs were extracted for each 1-min window of time with 50% overlaps, resulting in one time series (trajectory) per NP. Extracted NP trajectories were normalized per well and per window of time, and a moving median filter was applied to reduce outliers. A *k*-means trajectory clustering technique was used to find 10 clusters of trajectories for each NP and to assign cluster IDs to the trajectories of each compound. Then, for each compound, a vector with a total of 19 cluster IDs (one per NP) was assigned and used to classify the compounds as neuroactive or negative compounds using a Support Vector Machine (SVM) classifier. The entire classification model was trained with 73 compounds (42 neuroactives, 31 negatives) and yielded a classification accuracy of 93.2%. When using the model to classify the 384 compounds, 257 were identified as neuroactives and 127 as negatives. By comparison, when TI was excluded from the SVM classifier, classification accuracy of the same 73 neuroactive/negative compounds decreased to 86.3%. The higher classification accuracy of the SVM model that uses TI data demonstrates including TI is more effective for identifying acute neuroactive compounds when performing single-point screening. *This abstract does not reflect policy of the US EPA.*

2979 Updates to the Cramer et al. Decision Tree and Thresholds of Toxicological Concern to Improve Safety Assessment and Prioritize Chemicals for Testing

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The Cramer *et al.* (1978) Decision Tree (CDT) prioritizes chemicals according to their toxic potential using a sequence of 33 mainly structure-based yes or no questions, to which the answer either refers the user to another question or assigns the substance to one of three structural classes of toxic potential. Each question was designed based on information on chemical structure, reactivity, metabolism, toxicokinetics, biochemistry, and animal toxicology existing over 40 years ago. The concept of the Threshold of Toxicological Concern (TTC) refers to the establishment of a level of exposure for chemicals in each TTC class below which there would be no appreciable risk to human health. Each of the three CDT classes has a corresponding TTC level. Once a chemical is assigned to a CDT class, its consumption is assumed to be reasonably safe provided its intake is below its class TTC. Given the scientific knowledge accumulated since 1978, the CDT has been long overdue for an update. More than 18,000 scientific studies were reviewed to determine the effects of species, strain, sex, and target organ on toxicity and metabolic fate. These studies provided no-observed-effect-levels for 1,900+ substances that were then organized according to their structure, metabolic fate, and toxic potential. Analysis of this database resulted in the development of more refined questions, leading to a better separation between classes and an increased number of classes of toxic concern along with a broader scope of chemicals that are addressed by the Expanded Decision Tree (EDT). The toxic potential of each of the six classes was quantified by determining the EDT class TTCs. During the last seven decades, scientific advancements have led to an exponential increase in the number and types of chemicals to which humans are exposed, leading to an ever increasing need to screen and prioritize these substances according to their relative toxicity. Furthermore, calls for reducing the use of animals for safety testing has been increasing. Hence, updating of the scientific underpinnings of the CDT and TTC is timely and their expansion to allow for the screening of a broader scope of substances present in food, food contact materials, cosmetics, dietary supplements and elsewhere is desirable. This session will provide insight into the current use of the CDT and TTC, efforts to modernize them, and to increase their applicability.

2980 A Baseline Measurement for Endpoint Consistency across Zebrafish Laboratories

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Toxicology as a discipline is moving toward increased standardization of methods for testing and reporting end points in model organisms for improved, cross-laboratory data integration. Labs engaged in high-throughput analyses have established handbooks to internally standardize results, but these standards are rarely applied across laboratories. This poses a problem for integrating and comparing data across labs, which could support more robust corroboration in the confidence of results from toxicological assessments. To set a baseline, an informal survey was created asking participants to score 24 lateral images of zebrafish larvae for chemical-induced malformations (i.e., abnormalities) according to their laboratory practices and standards. Participants were allowed to answer using free text. Eighteen researchers from fourteen different labs participated. The free text results included 1748 terms which were mapped to 48 traits from the Zebrafish Phenotype Ontology (ZP) for comparison. Both the reported endpoints and the terms used to describe them were very heterogeneous and had little agreement - even the most straightforward of endpoints, normal and dead embryos,

were not consistently identified. Abnormalities of the heart and yolk were the most consistently identified (67% and 63% agreement, respectively) while abnormalities of the pectoral fin and the gut were least consistently identified (6% and 11% agreement, respectively). Abnormalities of the gut, pectoral fin, and otic vesicles were the most heterogeneously described while abnormalities of the trunk, snout, and heart were the most homogeneously described. Participants were able to score every image. All images were lateral views at stage 96 hours post fertilization (hpf) which were taken with the VAST (Vertebrate Automates Screening Technology) System. It is clear from these results that the community requires improved standards for defining and scoring toxicological endpoints. Future work will include conducting a study that includes a controlled vocabulary with textual definitions for scoring embryo images, to determine if this improves consistency.

PS 2981 High-Throughput Phenotypic Profiling (HTPP) to Discern Putative Mechanism of Action (MOA) for Environmental Chemicals

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HTPP is an imaging-based profiling method that combines automated microscopy and image analysis to measure a large variety of morphological features at the single cell level. The USEPA is currently exploring the use of HTPP for rapid bioactivity screening and hazard characterization of environmental chemicals. Here, we investigate the use of HTPP data for identifying chemicals with similar bioactivity profiles and discerning putative MOA. We screened a set of 120 reference chemicals to compare against 462 environmental chemicals selected from the ToxCast collection. Human U-2 OS cells were plated in 384-well format and treated with 8 concentrations (0.03 - 100 µM) of test chemicals. At 24 h, cells were fixed and labeled with fluorescent probes to visualize multiple organelles, including nucleus, nucleoli, endoplasmic reticulum, golgi, cytoskeleton, plasma membrane and mitochondria. Confocal images were acquired and 1300 phenotypic features were extracted at the single cell level. Cell-level data was normalized to the median and variance of the solvent control and aggregated to a well-level median. Well-level data was averaged across replicates (n = 4). Profiles for each chemical were obtained by retaining the largest effect size observed at non-cytotoxic concentrations. Signatures were computed by flooring values < 1.5 to 0 and biological similarity of chemicals was evaluated using Pearson correlation. Structural similarity was evaluated by comparing Morgan fingerprints using Tanimoto similarity. Within the reference chemical set, 16 distinct profile clusters were observed. Reference chemicals with various DNA damaging mechanisms clustered and demonstrated similarity with a subset of environmental chemicals. Different classes of pesticides also had distinct profiles. Five structurally-related organochlorine pesticides affected DNA texture and had biological similarities between 0.5 and 0.7. A group of strobilurin fungicides had similar profiles based on changes in mitochondrial features, consistent with the ability of strobilurins to inhibit fungal mitochondrial respiration. In summary, HTPP facilitated identification of chemicals with similar bioactivity profiles and potentially similar MOA, both among structurally-related and structurally diverse chemicals. *This abstract does not reflect US EPA policy.*

PS 2982 Quantitative Integration of Dose-Response Data for Relative Potency Estimates of Dioxin-Like Chemicals

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Toxic equivalency factors (TEFs) for dioxin-like chemicals (DLCs) are informed by an underlying database of relative potency (REP) estimates composed of heterogenous data from various study types, species, duration, dose, methods for deriving REPs, etc. The objective herein was to update the REP database and combine these heterogeneous data to infer quantitative dose-response (DR) distributions of REPs for each of 28 DLCs. Using a systematic search approach, > 50 studies were added to the database, doubling the size to >1200 datasets with DR and/or REP data. Individual DR curves were fitted to each dataset with DR data. Then, each DR curve was standardized by normalizing to the reference DR curve for each dataset. For each congener, from the set of study-specific standardized DR curves and/or study-specific REP values, an "average" standardized DR curve was inferred using a hierarchical Bayesian model. By comparing each congener-specific average standardized DR curve to the TCDD average standardized DR curve, a TEF value for each congener was inferred, along with uncertainty in the inferred value. Quantitative weighting based on study quality was incorporated by modeling increased heterogeneity of study-specific DR curves for lower-quality studies. For many data-rich congeners (e.g. 2,3,4.7,8-PeCDF), predicted TEF values are on the order of 2005 WHO TEF values; TEF 95% credible intervals are less than 1 order of magnitude wide. For congeners with little data (e.g. 1,2,3,7,8,9-HxCDF), TEF 95% credible intervals are 2 or more orders of magnitude wide. By modeling standardized DR curves, our approach harmonizes heterogeneous DR and REP data, and allows full consideration of shape and parallelism of DR curves — collectively demonstrating integration of dose-response data across study types and endpoints, including quantitative consideration of individual REP quality and relevance.

PS 2983 EU-ToxRisk Guidance for New Approach Methods (NAM)-Supported Read-Across

EU-ToxRisk Guidance for New Approach Methods (NAM)-Supported Read-Across

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Read-across (RAX) is a valuable approach to fill data gaps for complex toxicological endpoints without the use of *de novo in vivo* studies. Toxicological data from compounds with certain structural/physicochemical properties can be extrapolated to similar compounds that lack these data. For RAX to be reliable, however, biological similarity has to be accounted for as well. NAMs that describe toxicokinetic and toxicodynamic properties can be deployed to verify this biological similarity, and to establish a scientifically reliable and robust RAX. The use of NAMs goes hand in hand with new challenges like scope of *in vitro* testing, use of AOPs, interpretation of conflicting results, description of uncertainty, and reliability of test methods. More guidance is needed to assure transparent and accurate use of NAMs and by this promote acceptance of these new data within RAX assessments. In May 2019, the Horizon 2020 European collaborative project EU-ToxRisk - An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century - together with European agencies (ECHA, EFSA, SCCS), US agencies (NTP, EPA), and global organizations (OECD), hosted the workshop "New Approach Method (NAM)-supported read-across: from case studies to regulatory guidance in safety assessment". Based on case studies that targeted different regulatory applications, the use of NAMs was discussed with a special focus on the regulatory context and the associated regulatory requirements that these approaches have to fulfil. Furthermore, some of the cases have also been reviewed by the OECD IATA working group. This poster highlights the main learnings from the feedback on the NAM-based read-across reports, such as the requirements for data and method descriptions, the application of AOPs in terms of IATAs, the use of positive and negative controls to proof fitness-for-purpose of testing strategies, and uncertainty assessment.

PS 2984 Physiologically Based Pharmacokinetic Modeling of Impact of Nonalcoholic Fatty Liver Disease on Toxicokinetics of Perchloroethylene in Mice

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Nonalcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease in the Western population and steadily increasing in worldwide populations with metabolic syndrome, which is becoming more common due to the Western diet. The physiological changes associated with the disease substantially affect chemical toxicokinetics, which in turn modulate chemical toxicity. This study aims to use physiologically-based pharmacokinetic (PBPK) modeling to characterize the impact of diet-induced NAFLD on toxicokinetic variability of perchloroethylene (PERC) in mice. Quantitative measures of physiological and biochemical changes associated with the presence of NAFLD induced by high-fat or methionine/choline-deficient diets in C57B1/6J mice are incorporated into a previously developed PBPK model for B6C3F1, Swiss, and C57B1/6J male mice on normal diets. For instance, C57B1/6J mice having various stages of diet-induced NAFLD showed changes in fat and liver volumes and blood:air and liver:air partition coefficients, which are incorporated into the model. Hierarchical Bayesian population analysis using Markov chain Monte Carlo simulation is conducted to characterize uncertainty and inter-diet and inter-strain variability in PERC toxicokinetics and metabolism. The updated population model accurately predicts *in vivo* toxicokinetics of PERC oxidative and glutathione conjugative metabolites in all tissues across all diets and strains of mice. Parent compound toxicokinetics is well-predicted

in healthy mice, but model predictions of PERC toxicokinetics in blood and fat are underestimated in mice fed NAFLD-inducing diets. Toxicokinetic variability across strains and diets is evident, with mice on NAFLD-inducing diets showing greater oxidative and lower conjugative metabolism as compared to healthy mice of all strains. Moreover, the variation across three diets in C57B1/6J mice is at least as great as the variation across three strains on normal diets. These results suggest that non-genetic factors such as diet and pre-existing disease conditions may be at least as influential as genetic factors in altering chemical toxicokinetics, and thus are likely to be a substantial contributor to population variation in susceptibility to chemical exposures. *This work was supported by a grant from NIH/NIEHS (P42 ES027704).*

2985 Exploring the Use of Compound-Induced Transcriptomic Data Generated from Cell Lines to Predict Compound Activity toward Molecular Targets

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Pharmaceuticals or phytopharmaceuticals molecules rely on the interaction with one or few specific molecular targets to induce their anticipated biological responses. Nonetheless, these compounds also are prone to interact with many other biological targets, also known as off-targets. This fact is particularly critical when some (or a combination) off-targets are associated to adverse effects. Unfortunately, off-target identification is extremely difficult and expensive. Consequently, QSAR models predicting the activity of a molecule on a particular off-target have gained importance in the de-risking process of chemicals. For example, QSAR models have been designed to predict the risk associated with mutagenicity, oral acute toxicity or endocrine related toxicity. However, only few off-targets are well characterized and with enough data to build such *in silico* models. A good alternative to individual off-target evaluations is the use of integrative evaluations such as transcriptomics obtained from compound-induced gene expression derived from *in vitro* cell cultures. The advantage of this strategy is to inform about the consequences of the interaction of compounds on many possible molecular targets and biological pathways, without having any constraints concerning the chemical space. In this work, we assess the value of compound-induced transcriptomic data to build machine-learning models that can predict molecular targets (or off-targets) causally associated to known toxicity. For this, we trained random forest models using data from the cMAP L1000 dataset, which contains a large number of compound-induced gene expression profiles. Transcriptomic-based machine learning models were able to predict off-targets using data generated from appropriate cell lines; even in some cases, outperforming QSAR models. We also provide a simple framework to determine in which cases the use of transcriptomics data exploring biological spaces can help to overcome the limitations derived from a restricted chemical space.

2986 A Deep Generative Model Predicts Tissue Response to Chemical Perturbation

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Predictive computational toxicology plays an essential role in safety testing and has the potential to reduce animal usage. A central goal of predictive computational toxicology is to predict tissue responses to perturbation across domains, such as doses, tissues, organs, or species. In contrast to mechanistic, pathway-based models, the recent rise of deep generative modeling techniques provides an alternative approach to modeling the underlying structure of experimental data. Here we have developed a deep learning-based variational autoencoder model to predict dose responses of the mouse liver to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) perturbation using transcriptomic datasets. After training the model with liver transcriptome data from control groups, the model was used to encode transcriptome data into a compressed latent space. Following vector arithmetic operations in latent space, the results were decoded back to transcriptome space as the predicted response. We demonstrated here that the variational autoencoder can predict responses of new tissue samples to various doses of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) perturbation. Deep generative computational models can potentially add to the set of predictive toxicology tools and help reduce the need for expensive *in vitro* and *in vivo* experiments.

2987 An Open-Source *In Silico* Prediction Model of Hepatic Intrinsic Clearance for *In Vitro* to *In Vivo* Extrapolation

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Chemical risk assessment is moving away from animal testing with the goal of incorporating predictive approaches, including computational modeling and simulation into risk assessment. A tiered testing approach has the potential to provide new risk assessment information within lower tiers of testing focused on rapid prioritization of chemicals using high-throughput techniques. Dosimetry, by determining the amount, rate, and distribution of a chemical in the body, is thus essential for the use of new approach methodologies in risk-based decision making. Use of models such as high-throughput *in vitro* to *in vivo* extrapolation (HT-IVIVE) has allowed dosimetry considerations to be more readily incorporated earlier in safety decision making processes. To keep pace with *de novo* computational approaches for estimating hazard, new tools are needed that can provide reasonable estimates of chemical clearance without requiring wet-lab experimentation and analytical chemistry. To this end we have developed a free tool using published intrinsic clearance data that uses physicochemical information to estimate intrinsic clearance. This tool allows a user to input a novel structure by hand or a series of structures using a chemical structure file such as a SMILES or SDF file. A prediction is then generated using a variety of machine learning algorithms along with a confidence metric. The descriptors used in our model are structural as well as predicted physical chemical. In our initial development, the most successful algorithms were the neural network and nearest neighbors algorithms. In the case of the nearest neighbors, this tool also displays structurally similar neighbors from the training set. A total of 433 compounds were used in training set and 100 as test set. Predictions on an independent test set showed that our best performing models could predict the clearance value within one log unit of the known value 65% of the time, and 81% were within one and a half log units of the known value.

2988 An Interactive Online Database for Mining Information on Tobacco Products

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Our lab has obtained large amounts of data on flavor chemicals and metals in electronic cigarette fluids and aerosols, the cytotoxicity of these products, and the specific ingredients that cause cytotoxicity using various assays and cell types, including stem cells. In order to manage, search, and work with these data and make them available to others in a useful form, we have developed a searchable online database. The centralized database is being created using Microsoft SQL Server 2017, which is an intelligent relational database management system that runs on the SQL programming language. The SQL Server was chosen since it optimizes the storage of data and ensures security, integrity, and consistency. SQL also gives various ways to present the data analytically, making it convenient for a user to mine and extract useful information. A layout of the database was designed to store data in tabular form with appropriate naming conventions and necessary columns. The master tables are formed by identifying and assembling subjects from experiments and each tabular row is assigned a unique ID. These unique IDs help distinguish subjects across the database and serve as foreign keys to join related tables to retrieve complex information. The database has an interactive web interface that can be leveraged by users worldwide to perform various functions that include searching on keywords (e.g. specific metals and elements, specific flavor chemicals, product names, cell types), visualizing graphs, constructing statistical tables, and navigating to the published papers. Multiple cell types have been used in the cytotoxicity experiments enabling direct comparisons between the responses of stem cells and differentiated cells. The database is scalable and additional information can be added to the database as it becomes available, by conveniently importing and exporting data and modifying the database schema to add new columns and constraints.

2989 Robust Workflows for the Analysis of High-Throughput Transcriptomics Data in Chemical Safety Screening

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High-Throughput Transcriptomics (HTTr) is emerging as a chemical toxicity screening method that allows for broad assessment of many target pathways and modes of action in a single assay, which can inform both hazard identification and potency estimation. Steady decreases in cost have made HTTr feasible for screening hundreds to thousands of chemicals, while capturing expression profiles of over 10,000 genes per sample. To date, US EPA has generated HTTr screening data using the TempO-seq platform and MCF-7 cell line for ~2,200 chemicals, and is actively expanding this screening effort into additional cell types and chemicals. Batches of chemicals were tested in 8-point concentration series in triplicate using a 384-well plate format, with multiple types of reference samples per plate for evaluating technical and biological variability. The resulting data is substantially more complex than previous univariate high-throughput screening assays in terms of dimensionality, noise, and correlation structure. Thus, critical open questions remain regarding best practices for HTTr data analysis. Our recent work on this growing collection of data has included: 1) development of an open-source pipeline for rapid and robust processing of TempO-seq data; 2) comparison of multiple analytical methods for estimating differential expression and dose-response models; 3) quantification of reproducibility across platforms and methods; and 4) pathway-level analysis methods to summarize results and link findings to interpretable biology and known hazards. Specifically, we have developed novel quality control metrics to ensure accurate and reproducible hit calls and point-of-departure estimates. We have also rigorously analyzed reference samples across our screening efforts to demonstrate the overall reliability of the TempO-seq platform and quantify uncertainty resulting from multiple sources of experimental variation. We have tested multiple dose-response modeling approaches, including BMDExpress2 software and a novel pipeline that combines DESeq2 differential expression analysis with the ToxCast Pipeline (tcpl). Notably, the results show that aggregating signal at the pathway level improves reproducibility and reduces uncertainty in screening results. *This abstract does not necessarily reflect US EPA policy.*

2990 Deciphering Heterogeneity in Hepatic Responses to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Mouse Using Single-Nuclei RNA Sequencing

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Differences in cell type, spatial organization, and epigenetic regulation in a tissue can affect chemical- or drug-elicited responses. Bulk RNA-sequencing (RNAseq) involving tissue homogenization does not distinguish between cell-specific responses and only provides an average gene expression response across all cell types in a tissue. In contrast, single-cell RNA sequencing (scRNAseq) can assess cell-specific responses within the context of its resident tissue. We performed single-nuclei RNA-sequencing (snRNAseq) using nuclei isolated from frozen liver samples from male mice gavaged with sesame oil or 30 µg/kg TCDD every 4 days for 28 days. Approximately 17,921 expressed genes were detected across 16,711 sequenced nuclei isolated from control and TCDD treated samples. Overall, 11 cell (sub)types were identified including distinct pericentral, midzonal, and periportal hepatocyte sub-populations. Cell type clusters from nuclei expression data were similar to published expression profiles from fresh liver single-cell RNA sequencing (scRNA-seq) datasets generated using diverse technologies. Notably, cell type annotation from scRNA-seq was predictive of snRNA-seq clusters. TCDD treatment altered the proportion and composition of hepatic cell types was changed in response to TCDD treatment. Specifically, macrophage representation increased from 0.6% to 23.5%, while neutrophils were only evident in treated samples. The number of differentially expressed genes in each cell type ranged from 178 (B-cells) to 1,212 (pericentral hepatocytes). AhR battery genes such as *Cyp1a1*, *Cyp1a2*, and *Tiparp* were induced 8.8-, 10.5-, and 8.6-fold, respectively, in hepatocytes and endothelial cells. Other differentially expressed genes reported in bulk RNAseq analyses were also found to be specific to a cell type such as the 21.6-fold induction of *Gpnmb* only in macrophages, and the ~3-fold induction of *Col1a2* and *Col1a3* only in stellate cells. Functional analysis identified the hepatocyte-specific repression of oxaloacetate and bile acid metabolism, and the enrichment of B- and T-cell IL-7 signal transduction. Overall, snRNA-seq can be used to investigate cell-specific responses to TCDD within the context of whole tissue using frozen samples. *Funded by NHGRI R21HG010789.*

2991 Mechanism of Action Prediction Using High-Throughput Transcriptomics

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High-throughput transcriptomics (HTT) has been proposed as alternative cost-effective screening approach that covers a vast array of biological space of expression responses to chemical exposure. The Division of the National Toxicology Program (DNTP) is currently evaluating HTT as an approach to provide estimates of chemical exposure that may pose minimal human risk. HTT was evaluated in a 5-day (repeated dose) rat model with the objective to study and characterize the transcriptional changes in the liver and kidney. This approach will be useful to prioritize chemicals for further testing while providing actionable data in a timely and cost-effective manner. Twenty chemicals, most having been tested by the NTP in 2-year bioassays, were chosen for this study. The 20 chemicals are Acrylamide, Bisphenol AF (BPAF), Bromodichloroacetic Acid, Coumarin, Di(2-ethylhexyl) phthalate (DEHP), Pentabromodiphenyl Ether Mixture (DE-71), Ethinyl Estradiol, Fenofibrate, Furan, Ginseng, Hexachlorobenzene, Methyl Eugenol, Milk Thistle Extract, Perfluorooctanoic Acid (PFOA), Pulegone, Triclosan, Tris(chloropropyl)phosphate (TCPP), Tetrachloroazobenzene, Thujone and Tetrabromobisphenol A. Male Sprague Dawley rats were exposed daily for 5 consecutive days by oral gavage to 8 to 10 dose levels of each chemical. Liver and kidney were collected 24 hours after the final exposure for HTT using the Biospyder rat S1500+ platform. Using GENIE's network analysis module we (1) find groupings within the 20 chemicals and (2) perform transcriptomics read across to predict the mechanism of action (MOA) of each chemical. We obtained the following clear groups within the chemicals: Group #1 - PFOA, Fenofibrate, DEHP and Triclosan, Group #2: BPAF and ethinyl estradiol, Group #3: Coumarin and TCPP, Group #4: Pulegone, Furan and Methyl Eugenol. The rest of the chemicals do not group with any other chemical in the study. GENIE Network analysis module was used to compare the gene expression profile of each of these chemicals to thousands of gene expression signatures compiled from large curated databases such as Drugmatrix and TG-GATES rat database. For each of these chemicals, the top hits from the GENIE Rat database match the known MOAfor the chemical. For example, all the top hits with pvalue<0.005 for PFOA and Fenofibrate in this study are fibrates (bezafibrate, fenofibrate, pirinixic acid, clofibric acid, gemfibrozil etc.) in the Drugmatrix/TG-GATES database. This illustrates that proposed approach is applicable to any chemical of interest for MOA characterization.

2992 Physiologically Based Pharmacokinetic Modeling Improves *In Vitro* to *In Vivo* Extrapolation of Transcriptional Points of Departure

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In vitro (cellular) approaches are promising alternatives to current *in vivo* methods for chemical risk assessment. In particular, transcriptome-based *in vitro* methods could provide a powerful tool for regulatory decision-making. However, the use of such models brings new challenges associated with translating *in vitro* concentrations to corresponding *in vivo* internal exposures. Physiologically-based pharmacokinetic (PBPK) modeling provides an effective framework for conducting quantitative *in vitro* to *in vivo* extrapolation. Given that previous results suggested apical and transcriptomic endpoints show similar points of departure (POD) *in vivo*, we tested the hypothesis that an *in vivo* transcriptional POD could be accurately predicted from an *in vitro* POD using PBPK modeling. Transcriptional profiles from rat liver samples and rat primary hepatocytes exposed to different chemicals from the Toxicogenomics Project-Genomics Assisted Toxicity Evaluation system (TG-GATEs) database were used to generate transcriptome PODs using BMDExpress software. Because *in vitro* concentrations may not accurately reflect *in vivo* internal exposures, we integrated PBPK software (GastroPlus) to calculate blood Cmax and liver Cmax from *in vivo* data using measured or predicted plasma protein binding and hepatic clearance data. Our findings suggest a stronger correlation between *in vitro* and *in vivo* transcriptional PODs using measured plasma protein binding and hepatic clearance data instead of GastroPlus-predicted values (R^2=0.45 vs R^2=0.08). These findings suggest that *in silico* PBPK modeling may improve the prediction of *in vivo* PODs from *in vitro* transcriptome data.

2993 Whole Transcriptome Extrapolation, Mechanism of Action Analysis, and Network Visualization: Tunicamycin Case Study

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Targeted transcriptomic analyses decrease the time and resources associated with obtaining whole transcriptomic data in high throughput screens. The TempO-Seq S1500+ platform(s), now available for human, mouse, rat and zebrafish, measures a carefully selected subset of the transcriptome such that it provides adequate representation of the biological responses, functional pathways and coregulate gene modules in the transcriptome. While measurement of these S1500+ genes provides a valuable assessment of gene expression activity, extrapolating expression values to the whole transcriptome (~26 K genes in humans) increases the power of pathway analysis algorithms. Here we use a human S1500+ Tunicamycin dataset to explore the improved downstream analysis achieved by extrapolating expression to the remaining transcriptome. Extrapolation increased the number of significant genes by 49%, bringing to the forefront many genes and pathways that have been established to be differentially expressed by Tunicamycin exposure without significantly changing the sample variability. We demonstrate that gene- and pathway-level biological interpretations were improved by extrapolating from the ~3K measured genes to approximately 26K genes before performing a variety of downstream applications, including differential expression analysis, gene set enrichment pathway analysis, and DAVID keyword analysis. The extrapolated data highlight the role of metabolism/metabolic pathways, the endoplasmic reticulum, and the unfolded protein response, each of which are key activities associated with Tunicamycin exposure that were underrepresented prior to extrapolation. Furthermore, when including extrapolated genes, Tunicamycin rose from third to first upstream regulator in Ingenuity pathway analysis, from sixth to second most correlated compound in Nextbio analysis, and Tunicamycin exposure is identified as top three signatures in GeniE Network Analysis. Our case study suggests that whole transcriptome extrapolation of data from the S1500+ platform provides improved insight into biological mechanisms and functional outcomes.

2994 Expression of Copper Transport Protein-2 (CTR2) in the Blood-CSF Barrier: Effect of Lead Exposure *In Vitro*

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Copper (Cu) is an essential metal for living organisms; either deficiency or overload of Cu can cause cellular dysfunctions. Thus, the Cu homeostasis is tightly regulated by multiple copper transport proteins. Copper transport protein-2 (CTR2) has recently been suggested to regulate the function of copper transport protein 1 (CTR1) so to maintain the stable intracellular Cu level. However, the existence of CTR2 in the blood-cerebrospinal fluid (CSF) barrier (BCB) in brain choroid plexus, which regulates material movements between the blood and CSF, was virtually unknown. This study was designed to explore the existence of CTR2 in the BCB; furthermore, the effect of *in vitro* exposure to lead (Pb), a toxic metal abundantly accumulated in the choroid plexus, on the expression of CTR2 was investigated. Immortalized choroidal epithelial Z310 cells and freshly isolated mouse plexus tissues were used to identify the expression of CTR2. Both qPCR and confocal immunofluorescent data demonstrated the presence of CTR2 in Z310 cells as well as in plexus tissues; in Z310 cells, CTR2 appeared to be localized in cytoplasm and stored in vesicles, while in plexus tissues, CTR2 signals were primarily present in the cytosol between nuclei and brush boarder facing the CSF. When Z310 cells were incubated in a Pb-containing medium at 2, 5, and 10 μM for 24 hrs, the levels of CTR2 expression, as quantified by immunofluorescence, were reduced by 35.50%, 37.14%, and 52.10%, respectively, as compared to controls ($p<0.05$, $n=4$). The confocal data also revealed that incubation of plexus tissues in a Pb-containing artificial-CSF significantly reduced the fluorescent signals associated with CTR2. Moreover, CTR2 signals in Pb-treated plexus tissues appeared to be concentrated in areas beneath the epithelial microvilli, suggesting an intracellular trafficking of CTR2 towards the CSF-facing epithelial surface. These observations provide the first-hand evidence that CTR2 exists in the blood-CSF barrier, and Pb exposure not only reduces the expression of CTR2, but also causes it to translocate toward the epithelial microvilli. Mechanistic studies to investigate the function of CTR2, in coordination with CTR1, in regulating Cu transport by the BCB, and the consequence of Pb exposure are currently in progress. *Supported in part by NIH/NIEHS R01 ES027078.*

2995 Neuroprotection by Luteolin and Gallic Acid against Cobalt Chloride-Induced Behavioral, Morphological, and Neurochemical Alterations in Wistar Rats

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Cobalt (Co) intoxication arising from occupational exposures and ion release from metal implants has been associated with neurological alterations such as cognitive decline, incoordination and depression. The present study evaluated the mechanisms of neuroprotection exerted by Luteolin (Lut; 100 mg/kg) and Gallic acid (GA; 120 mg/kg) in Wistar rats exposed to cobalt chloride (CoCl2) at 150 mg/kg for 7 consecutive days. Results indicate that CoCl2 induced neuro-behavioural deficits specifically by decreasing exploratory activities of CoCl2-exposed rats, increased anxiety, as well as significant reduction in hanging latency. Co-treatment with Lut or GA,however, restored these parameters to values near those of normal controls. Moreover, Lut and GA prevented CoCl2-induced increases in hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and nitric oxide (NO) in the brain, while also restoring the activities of acetylcholinesterase, glutathione-S-transferase (GST) and superoxide dismutase (SOD). In addition, Lut and GA produced significant reversal of CoCl2-induced elevation in levels of serum Interleukin 1 beta (IL-1β) and Tumor necrosis factor (TNFα). Meanwhile, immunohistochemistry revealed increased astrocytic expression of glial fibrillary acidic protein (GFAP), with intense calbindin (CB) D-28k staining and pronounced dendrites in the Purkinje cells. In contrast, the CoCl2 group was characterized by decreased number of neurons expressing CB and dendritic loss. Taken together, mechanisms of luteolin and/or gallic acid protection against Co toxicity involved restoration of Ca2+ homeostasis, acetylcholinesterase and antioxidant enzyme activities, as well as inhibition of lipid peroxidation in the brain.

2996 Effects of Methylmercury on Excitatory Amino Acid Transporters Expression in Cortical Astrocytes and Motor Neuron-Like Cells

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Excitatory amino acid transporters (EAATs) have the important role of removing extracellular glutamate from the synaptic cleft. They have been characterized as critical in protecting neurons against glutamate-induced excitotoxicity. Methylmercury (MeHg) is an environmental neurotoxicant identified as excitotoxic which induces excessive release of glutamate. MeHg induces EAAT impairment by reactive oxygen species generation. Consequently, this disrupts glutamate homeostasis, glutathione synthesis (an important antioxidant for toxicity) and eventually induces excitotoxic neuron death. EAAT1 and EAAT2 are primarily expressed in astrocytes and EAAT3 in neurons. Because of the established importance of EAATs, we hypothesized that MeHg exposure could exacerbate expression of EAATs in both astrocytes and motor neuron-like cells (NSC-34). To assess the effects of MeHg in the transporters, cortical astrocytes, extracted from mice, and NSC-34 cells were cultured and exposed to 1, 2 and 5μM MeHg for 3 hours. The capacity of EAAT expression after MeHg exposure, was examined using immunocytochemistry and the images were analyzed by the mean fluorescence intensity to quantify the expression of the proteins. We observed that MeHg induced EAAT3 overexpression which increased with the increases in the concentration of exposure. In addition, cortical astrocyte EAAT1 and EAAT2 expression were not affected by MeHg after 3 hours of exposure. Modulating EAATs function may be a therapeutic target to attenuate excitotoxicity-induced pathogenesis in diseases such as Amyotrophic Lateral Sclerosis. *This research was supported by NIH grant R01ES024064, and R25NS090989- NINDS ENDURE Program, and the Bridge to the Ph.D. in Neuroscience Program.*

2997 Methylmercury-Induced Excitotoxcity in Forebrain Astrocytes and Pharmacological Mediation by ω-conotoxin GVIA and Nimodipine

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Methylmercury (MeHg) is a contemporary neurotoxicant that affects both neurons and supporting glial cells including astrocytes. In the Amyotrophic Lateral Sclerosis (ALS) humanized transgenic mouse model, which over-expresses the human superoxide dismutase1 mutant- SOD1 G93A, MeHg ex-

posure hastened the onset of ALS-like phenotype. On a cellular level, MeHg toxicity causes a spontaneous release of glutamate, which then increases intracellular [Ca2+], damages mitochondria, causes reactive oxygen species formation, and ultimately leads to cell death. Calcium channel blockers have been shown to delay the onset of MeHg-induced increases in intracellular [Ca2+], and reduce the incidence of cytotoxicity induced by MeHg. In this study, we used astrocytes derived from SOD1G93A mice to test for the contribution of voltage-gated Ca2+ channels to cytotoxicity induced by MeHg. In particular, antagonists of the N-type (Cav2.2) and L-type (Cav1.3) channels were tested, as these types of Ca2+ channels have been reported to exist in astrocytes. In effort to mediate MeHg-induced cytotoxicity, calcium channel blockers, ω-conotoxin GVIA (which blocks N-type) and nimodipine, (which blocks L-type channels) were used as a pretreatment of isolated astrocytes from SOD1 mice which were then exposed to 2μM and 5μM MeHg. A calcein viability assay and fluorescence microscopy was performed to analyze the extent of protection that the calcium channel blockers provided to the astrocytes. Viability percentages were calculated from average cell counts from control and experimental assays. It was hypothesized that treatments of ω-conotoxin and nimodipine would reduce extracellular Ca 2+ from entering the cell. Results concluded that the calcium channel blockers increased cell viability with the lower concentrations of MeHg (1, 2μM), but lost effectiveness at 5μM MeHg. Thus voltage-gated Ca2+ channels contribute to entry of Ca2+ inducing cytotoxicity in SOD1 mouse-derived astrocytes. *This research was supported by NIH grant R01ES024064, and R25NS090989- ENDURE Program, as well as an SOT Summer Toxicology Internship to MF.*

PS 2998 Neurotoxic Effect of Manganese and Vanadium Co-exposure in Animal Models of Parkinson's Disease

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Chronic environmental exposure to a mixture of two or more heavy metals, often from occupational or industrial sources, has been implicated in Parkinson's disease (PD). Vanadium pentoxide (V_2O_5) typically presents along with manganese (Mn), especially in welding rods. The neurotoxic consequences of chronic Mn/V_2O_5 co-exposure on key pathological features of PD such as α-synuclein protein aggregation remains to be investigated. In this study, we examined the neurotoxic effects of Mn/V_2O_5 co-exposure in a transgenic animal model of PD. Wild-type mice (naïve C57 black strain) and transgenic mice expressing A53T alpha-synuclein were intranasally co-exposed to 100 μg $MnCl_2$ and 75 μg V_2O_5 five times a week, representing a 5-day work week of occupational exposure, for up to three months, and all mice were subjected to a battery of behavioral tests at monthly intervals. Exploratory locomotor activity in an open-field test declined significantly in Mn/V_2O_5-treated A53T male mice and female mice, but not in Mn/V_2O_5-treated C57 black mice. Motor coordination during forced locomotor activity, in contrast, was not significantly affected by Mn/V_2O_5 treatments in either A53T or C57 black mice. Effects of chronic intranasal co-exposure to Mn/V_2O_5 on olfactory discrimination of social cues were significant starting the second-month post-treatment. Mn/V_2O_5-treated A53T mice and C57 male mice exhibited olfactory deficits while C57 female mice did not. Treatment with Mn/V_2O_5 did not induce depression-like behavior in any group as measured by tail suspension or forced swim tests. Taken together, our results suggest that co-exposure to Mn and V_2O_5 can adversely affect α-synuclein-overexpressing A53T mutant mice when compared to naïve C57 black mice. These results highlight a possible role of neurotoxic metal mixtures in environmentally linked Parkinsonism *(DOD W81XWH1810106/CDMRP, NIH R01 ES026892, and Eugene and Linda Lloyd Endowed Chair).*

PS 2999 Altered Prefrontal-Hippocampal Network Activity following Chronic Early-Life Lead Exposure in Rats

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Childhood lead exposure affects millions of people and causes irreversible mental health problems that persist in adulthood. In particular, chronic early-life lead exposure (CELLE) is a major risk factor in schizophrenia. Likewise, CELLE models in rats show many of the behavioral, neurochemical, and neuropathological endpoints of schizophrenia. Importantly, CELLE animals show impaired pre-pulse inhibition (PPI) and reduced parvalbumin-positive GABAergic interneurons in the prefrontal cortex and hippocampus. However, the consequences of CELLE on prefrontal-hippocampal network states have not been examined. Here, we recorded local-field potentials in the dorsal hippocampus and the medial prefrontal cortex as rats freely explored or retrieved sporadically-delivered rewards in an open-field environment. In CELLE rats, we found altered synchronization in the delta (1-4Hz), theta (5-11Hz), and high gamma (65-120Hz) bands, which was related to behavior. These results parallel what is reported in other rodent models of schizophrenia and may correspond to irregular EEG patterns found in schizophrenic patients. We also observed sporadic episodes of strong nonconvulsive absence seizures in CELLE rats, which were eliminated with the administration of ethosuximide (ETX, 50-200 mg/kg), a first-choice drug for absence epilepsy. Together, our findings suggest that CELLE causes aberrant long-range interactions and excitatory-inhibitory imbalances within local circuits of the prefrontal-hippocampal system. Understanding the network mechanisms at play in CELLE will lay the foundation for developing diagnostic tools and therapeutic strategies in childhood lead exposure.

PS 3001 Oxidative Stress, Trace, and Toxic Metal Levels in Alzheimer's Disease in an African Population

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Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by loss of memory resulting from neurodegenerative disorders, this has been attributed to oxidative stress and accumulation of Amyloid (Aβ) protein in the brain. Environmental and genetic alterations have been implicated as the pathogenesis of the disease. This work investigated levels of selected trace (Iron, Zinc and Copper) and toxic (Cadmium and Lead) metals in AD patients. In this case-control study, a total of 38 participants aged ≥60years consisting of 18 clinically diagnosed with AD and 20 apparently healthy age-matched adults were recruited from the University College Hospital Ibadan Geriatric Centre. Semi-structured questionnaires were used to obtain demographic information, clinical history, lifestyle and dietary patterns from participants. Plasma levels of iron, copper, zinc and blood levels of lead and cadmium were analyzed using Atomic Absorption Spectrophotometry (AAS). Plasma levels of malondialdehyde (MDA), total antioxidant capacity (TAC), hydrogen peroxide (H_2O_2), and total plasma peroxide (TPP) were determined spectrophotometrically, while oxidative stress index (OSI) and copper to zinc ratio (Cu:Zn) were calculated. Mean plasma level of zinc was significantly lower in the cases (86.04±11.07μg/dl) compared to controls (108.80±12.47μg/dl), while blood lead (13.85±2.96μg/dl, 8.32±2.10μg/dl) and cadmium (1.34±0.71μg/L, 0.71±0.14μg/L) levels were significantly higher in cases than in controls respectively. Although Fe and Cu levels were similar in cases and controls, Cu:Zn ratio was significantly elevated in cases compared to controls (p=0.000). Though other OS markers were not significantly different in both groups, TPP was significantly higher in cases (64.96±7.20 μmol/H_2O_2 vs. 55.41±2.38μmol/H_2O_2) while MDA correlated inversely with TAC in cases (r= -0.477, p=0.045). The low plasma Zn coupled with high blood Pb and Cd levels may precipitate the elevated TPP and Cu:Zn ratio in cases. The reduced metallothionine defense of the system as indicated by the elevated Cu:Zn ratio in cases may also exacerbate this problem. Increased oxidative stress influenced by the high toxic metal level in cases may be participatory in the progression of AD.

PS 3002 Toxicogenomics for Understanding the Evolutionary Neurotoxicology of Manganese

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Manganese (Mn) is the twelfth most abundant element in the earth's crust. Mn is an essential trace mineral in nutrition that can be neurotoxic and neurodegenerative via environmental and/or nutritional acute and chronic exposure, especially during stages of development. Studies from our group, applying "omics" approaches, have revealed that environmentally relevant concentrations of Mn^{2+} (0.001-1.5 mM) disrupt metal homeostasis (K, Ca, Fe and Cu); protein metabolism, including translation, post-translation, and protein degradation as well as associated pathways such as those involved in energetics, cell signaling, cell cycle and neurotransmitter metabolism, in a suite of organisms, from yeast to human. Notably, these events during or after protein biosynthesis, have been associated with neurodegenerative disorders such as Alzheimer's disease, Amyotrophic Lateral Sclerosis, Parkinson's disease, and Huntington's disease. These findings suggest that disruption of protein biosynthesis is a key mechanism for Mn-induced neurotoxicity and neurodegeneration, but sometimes involving different genes or proteins. However, it is well known that from yeast to human, the *protein* translation is a complex, highly conserved and coordinated process mediated by the ribosome and some cases affected by metalloenzymes. Hence, for under-

standing eco-evolutionary dynamics, under the effects of manganese, the ionome-wide data and protein biosynthesis needs to be considered before drawing comparisons to humans.

3003 Genetic and Conditional Stimulation of Adult Neurogenesis Rescues Mice from Cadmium-Induced Impairments of Hippocampus-Dependent Learning and Memory

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Cadmium (Cd) is a heavy metal that has been recognized as one of the most toxic environmental pollutants. Because of its long half-life, Cd accumulates in human bodies and induces adverse effects in various organs. Cd is suggested to be a neurotoxicant. However, the full spectrum of its neurotoxicity is not fully understood. Our previous studies have found that Cd exposure can impair hippocampus-dependent memory, as well as adult hippocampal neurogenesis in mice. In order to establish a causal relationship between Cd-induced impairments of adult hippocampal neurogenesis and hippocampus-dependent memory, we conducted a rescue experiment to see if enhancing adult neurogenesis could rescue mice from Cd-induced decline of cognition. In this study, a group of a specific transgenic mouse (caMEK5) which allows inducible and conditional activation of adult neurogenesis upon tamoxifen treatment was exposed to 0.6 mg/L Cd through drinking water for 38 weeks. Behavior tests were conducted during the exposure period to investigate the effect of Cd on hippocampus-dependent learning and memory in mice. At 17 weeks into Cd exposure, impaired hippocampus-dependent spatial working memory was found in Cd-treated mice and then tamoxifen was administrated to increase adult hippocampal neurogenesis. At 6 weeks after tamoxifen treatment (31 weeks into Cd exposure), the spatial working memory in Cd-tamoxifen treated mice were recovered while the Cd-vehicle group still exhibited impaired spatial memory. In addition, after 38 weeks of Cd exposure, the hippocampus-dependent contextual memory was also impaired in Cd-vehicle treated mice, but not in other groups. We further examined the condition of adult hippocampal neurogenesis in caMEK5 mice after Cd and tamoxifen treatment by using immunostaining. There was a significant decrease in the total number of $BrdU^+$ cells and $BrdU^+$ $NeuN^+$ cells in the dentate gyrus of the hippocampus in the Cd-vehicle group, suggesting that Cd exposure impairs adult hippocampal neurogenesis in caMEK5 mice. However, we did not discover the similar effects of Cd in the Cd-tamoxifen group, which confirms that tamoxifen treatment recovered the impaired adult hippocampal neurogenesis. In addition, we found Cd exposure increased the brain Cd concentrations in caMEK5 mice. Overall, our study suggests that inhibition of adult hippocampal neurogenesis plays an important role in Cd-induced impairments of hippocampus-dependent learning and memory.

3004 Early-Life Lead (Pb^{2+}) Exposure Induces Transgenerational Effects on Zebrafish Brain Transcriptome

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Lead (Pb^{2+}) is a major public health hazard for urban children, with profound and well-characterized developmental and behavioral implications across the lifespan. The ability of early Pb^{2+} exposure to induce epigenetic changes is also well-established, which suggests that Pb^{2+}-induced neurobehavioral deficits may also be heritable across generations. Understanding the long-term and multigenerational repercussions of lead exposure is crucial for clarifying both the genotypic alterations behind these behavioral outcomes and the potential mechanism of heritability. To study this, zebrafish (*Danio rerio*) embryos (<2 hours post fertilization; EK strain) were exposed for 24 hours to waterborne Pb^{2+} at a concentration of 10 μM. The exposed F_0 generation was raised to adulthood and spawned to produce the F_1 generation, which was subsequently spawned to produce the F_2 generation. Previous avoidance conditioning studies determined that a 10 μM Pb^{2+} dose resulted in learning impairments persisting through the F_2 generation. RNA was extracted from control- and 10 μM Pb^{2+}-lineage F_2 brains, (n=10 for each group), sequenced, and transcript expression was quantified utilizing Quant-Seq. 648 genes were differentially expressed in the brains of F_2 lead-lineage fish versus F_2 control-lineage fish. Pathway analysis revealed altered genes in processes including nervous system development and function, neurotransmission, learning, conditioning, memory, endocrine system function, hormone regulation, and epigenetic modification, all of which are implicated in lead-induced neurobehavioral deficits and/or their inheritance. These data will inform future investigations to elucidate the mechanism of adult-onset and transgenerational health effects of developmental lead exposure.

3005 Lead Aggravates the Diabetic-Induced Neurodegeneration and Neuroinflammation and Neuro-Protecting Effect of *C. carandas*

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Diabetes, an unresolved metabolic disorder and lead contamination are prevalent problems in the contemporary society. Previously, reported suggest that either diabetes or lead exposure resulted in neurodegeneration in male rats. The aim of this study was to evaluate whether diabetic rats exposed to lead demonstrate a higher degree of neurotoxicity, inflammation and neurodegeneration when compared with lead-exposed control rats. And neuroprotective, anti-inflammatory and antioxidant activity of *C. carandas* constituents were also evaluated. Diabetes was induced by injecting single dose of streptozotocin (40 mg/kg body weight). Control and diabetic rats were exposed to lead through oral gavage for a period of 21 days and assessed for neuro-inflammatory markers and oxidative end points. Significant reduction in brain antioxidant enzyme activity, membrane proteins and ion channels including Acetylcholinesterase, Na^+-K^+ ATPase levels and glutathione levels were observed in diabetic rats with an elevation in levels of superoxide anions, hydrogen peroxides, lipid peroxidation. Mild histopathological malformations were observed in the brain of the diabetic rats. TNF-α, IL-6 transcripts and nuclear factor-κB expression were increased in the diabetic rat brain. Similar oxidative and neurotoxicity was observed in lead-exposed control rats. Further, lead exposed diabetic rats showed additional deterioration in hippocampal and inflammation end points and noteworthy elevation in oxidative toxicity suggesting that treatment with lead exacerbates neurotoxicity in streptozotocin-induced diabetic rats. The ameliorative efficacy of aqueous extract of C. Carandas was analysed in diabetic rat exposed to lead. Study shows the significant neuroprotective and anti-inflammatory activity of extract.

3006 Impact of Manganese Exposure on Mood in Welders

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Manganese (Mn) is an essential trace element found in many biological tissues. Yet, chronic exposure to Mn is neurotoxic and can lead to cognitive, psychiatric and motor deficits, with mood disorders being reported as one of the earliest symptoms in humans exposed to Mn. The goal of this study was to assess whether mood changes in welders exposed to Mn were associated with Mn exposure or neuroimaging markers, such as elevated brain Mn levels and thalamic γ-aminobutyric acid (GABA) levels, assessed by magnetic resonance imaging (MRI) and spectroscopy (MRS). Forty-five male welders were recruited from a local manufacturer. Each subject underwent an MRI/MRS exam to assess brain Mn deposition, as measured by the R1 relaxation rate, and thalamic GABA levels. The Brief Symptom Inventory (BSI) was administered to assess nine domains of mood, as well as a Global Severity Index (GSI). Individual exposure to Mn was assessed using personal air sampling and a work history questionnaire. Spearman Rank Tests were performed to test for associations between raw mood scores and R1 values, thalamic GABA levels and Mn exposure in the welders. Furthermore, the standardized t-scores for the mood values were compared against normative standards. Comparing the BSI t-score of the 9 mood categories including GSI, a summary score indicating the subject's distress level, against the male adult non-patient norm disclosed that 49% of the welders were classified as "clinical cases". The subdomains of somatization (SOM), obsessive-compulsive (OC) behavior and interpersonal sensitivity (IS) were significantly associated with elevated past year Mn exposure (SOM, IS: rho >=0.3, p< 0.05) and/or toenail Mn levels (SOM, OC: rho ≥ 0.3, p<0.05). Thalamic GABA levels were significantly associated with depression scores (rho=-0.44, p=0.02), and Mn levels of the Caudate Nucleus were found to be inversely associated with 5 of the 9 mood domains, as well as the overall GSI (all rho < -0.3, p< 0.05). In agreement with previous literature we report increased changes in mood and associations with increased Mn exposure in a typical occupational welding setting in the US with Mn exposure levels around 0.1 mg/m3. The association of depression scores with thalamic GABA is in line with reports of thalamic lesions being implicated with depression. However, the findings of an inverse relation to brain Mn levels will need to be investigated further. Factors, such as alcohol consumption and shift work will need to be considered as confounders.

3007 Methylmercury Exposure *In Vivo*-Induced Differential Effects on AMPA-Current in Brainstem Hypoglossal Motorneurons from Mice Expressing Wild-Type or the Mutant Human Superoxide Dismutase 1 (hSOD1) G93A Genes

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The exact causes of Amyotrophic lateral sclerosis (ALS), a progressive and fatal neurological disorder due to loss of upper and/or lower motoneurons (MNs), remain elusive. Gene-environment interactions are believed to be an important factor in the development of ALS. We previously showed that *in vivo* exposure of mice overexpressing the human superoxide dismutase 1 (hSOD1) gene mutation (hSOD1G93A; G93A), a mouse model for ALS, to environmental neurotoxicant methylmercury (MeHg) accelerated the onset of ALS-like phenotype. We also showed that mouse genotypes appear to affect the sensitivity of brainstem hypoglossal MNs to the MeHg-induced acute and chronic effects on neuronal excitability, synaptic transmission and AMPA receptor (AMPAR)-mediated currents *in vitro* and *in vivo*. Here we examined the time-courses of effects of MeHg on AMPAR-mediated currents in hypoglossal MNs in brainstem slices prepared from G93A, hSOD1wild-type (SOD1hWT) and WT mice following *in vivo* exposure to MeHg for defined time periods. Mice were exposed daily to 3 ppm MeHg via drinking water beginning at postnatal day 28 (PND28) and continued until PND47, 64 or 84, then acute brainstem slices were prepared and AMPAR-mediated currents were examined using whole cell patch-clamp recording technique. Brainstem slices of untreated littermates were prepared at the same time points to serve as control. AMPA-current was evoked at a holding membrane potential of -50 mV. MeHg exposure had no significant effect on AMPA-current in slices from SOD1hWT mice during any of those exposure time periods. MeHg also did not cause any significant effect on AMPA-current in G93A and WT hypoglossal MNs at PND47. However, at PND64 and 84, MeHg significantly increased the AMPA-current in hypoglossal MNs from G93A mice ($p<0.01$), whereas it significantly decreased the AMAP-current in hypoglossal MNs from WT mice ($P<0.05$). Therefore, MeHg exposure *in vivo* caused differential effects on AMPA-current in hypoglossal MNs from mice with different genetic backgrounds. MeHg appears to preferentially stimulate the AMPAR-mediated currents in G93A hypoglossal MNs in an exposure time-dependent manner, which may contribute to the AMPAR-mediated MN excitotoxicity, thereby facilitating development of ALS-like phenotype. *Supported by NIEHS/NIH grant ES024064.*

3008 BTBD9, a Restless Leg Syndrome-Associated Protein, Regulates Manganese-Induced Toxicity in *Caenorhabditis elegans*

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Restless Legs Syndrome (RLS) is a common neurological disorder seen in ~10% of the US population. RLS-associated sleep deprivation can seriously impact life quality, causing anxiety, depression and attention-deficit/hyperactivity disorder (ADHD) symptoms. Moreover, RLS may portend hypertension, heart disease and stroke. RLS exhibits both familial and non-familiar (idiopathic) forms, with ~60% of cases having a family history of the disease. BTBD9 is one of the genetic risk factors, associated with decreased serum iron (Fe) level. Interestingly, lymphocytes from RLS patients have an altered Fe management protein profile, which also regulates manganese (Mn) homeostasis. This raises the question as to whether the symptoms inherent to RLS patients are the result of Fe deficiency or elevated concentrations of another metal that opportunistically increases when Fe levels are low. Here we present novel data that BTBD9 functions to regulate Mn homeostasis in *Caenorhabditis elegans* (*C. elegans*). A blast search identified *hpo-9* as the BTBD9 homolog in *C. elegans*, with ~75% sequence similarity. A mutant strain tm3719 (*hpo-9*$^{-/-}$) carrying 761 bp deletion of *hpo-9* was obtained. We found that *hpo-9*$^{-/-}$ worms were more sensitive to Mn exposure. Upon Mn treatment, *hpo-9*$^{-/-}$ worms showed a significantly lower survival rate and more severe DAergic neurodegeneration compared with wild type worms. Interestingly, no difference was seen when worms were exposed to Fe. However, a low level of Fe (0.1 µM) pretreatment was able to protect Mn-induced lethality. To better characterize HPO-9 protein, a transcriptional fusion construct was created with green fluorescent protein (GFP) driven under *hpo-9* promoter. We found that GFP was present high in the head and pharynx and low in the intestine and seam cells. Using a confocal microscopy, we found that *hpo-9* was expressed in dopaminergic neurons, indicating that HPO-9 might play a role in dopamine signaling. To confirm that, we over-expressed HPO-9 in DAergic neurons of *hpo-9*$^{-/-}$ worms and found that it rescued Mn-induced DAergic neurodegeneration. Together, our results suggest a novel role for *hpo-9*/BTBD9 in regulating Mn homeostasis and possibly dopamine signaling in *C. elegans*. *Supported by NIEHS ES010563.*

3009 Modulating Effects of Resveratrol Supplementation on As_2O_3-Induced Neurotoxicity in Mice Hippocampus

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The ubiquitous distribution of arsenic makes it an environmental contaminant of global concern. Reported literature, identifies oxidative stress as key factor underlying inorganic arsenic (*iAs*) induced toxicity. Hence, the need of the hour is to identify safe therapeutic approaches for amelioration of *iAs* induced toxicity. The aim of the present study was to determine the ameliorative potential of resveratrol (antioxidant) on cognitive functions, oxidative stress, morphology and apoptosis in hippocampus (CA1) of female mice following arsenic trioxide (As_2O_3) exposure. Healthy adult female mice were randomly divided as control (normal; vehicle) and experimental groups receiving As_2O_3 alone (2, 4 mg/kg bw) or along with Resveratrol (40 mg/kg bw) by oral route for 45 days. The behavioral study (OFT, EPM and MWM) was carried out from Day 33-45. Perfusion fixed hippocampal tissues were processed for Cresyl violet and TUNEL staining whereas fresh hippocampi were subjected to biochemical estimation of reduce glutathione (GSH). As_2O_3 alone exposed animals showed dose-dependent enhanced anxiety levels and impairment of cognitive functions (learning and memory deficits) when subjected to OFT, EPM and MWM testing. Hippocampal GSH levels were found to be significantly down-regulated in As_2O_3 alone treated animals, thereby suggestive of As_2O_3-induced oxidative stress in these animals. Morphometric observations of Hippocampus (CA1) of As_2O_3 exposed group showed significant reduction in thickness of Stratum Pyramidale (SP), decrease in density and area of Pyramidal neurons. TUNEL study revealed intense dark brown staining of nuclei of pyramidal cells was observed in SP (CA1) of As_2O_3 alone treated animals. Co-treatment of resveratrol with As_2O_3 was helpful in restoration of these changes to a substantial extent. Our observations support modulating effect of Resveratrol against As_2O_3-induced cognitive dysfunctions, altered morphology, apoptosis and oxidative stress in female mice hippocampus.

3010 Nrf2 Knockout Causes Differential Effects of MeHg on Keap1 and Cystine/Glutamate Antiporter mRNA Expression in Mouse Primary Spinal Cord Astrocytes

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Methylmercury (MeHg)-induced neuronal degeneration involves multiple pathways which are to some extent cell-type dependent. Dysregulation of intracellular calcium homeostasis and glutamate transmission are common targets. Redox homeostasis is also involved; it is regulated by the Nrf2-ARE pathway. Nrf2-ARE pathway activation increases the expression of antioxidant genes such as glutathione (GSH) synthetase, GSH peroxidase (Gpx), the cystine/glutamate antiporter (system Xc-) and multidrug-resistant associated protein (Mrp). Astrocytes are also a target for MeHg. Astrocytic Nrf2-ARE pathway is more active compared to neurons. Thus, neurons require the antioxidants supplied by astrocytes, particularly during oxidative stress, and dysregulation of the Nrf2-ARE pathway in astrocytes could be detrimental to neurons. Perturbation of the Nrf2-ARE pathway has been reported in MeHg-induced toxicity in cortical and cerebellar astrocytes as well as neurons, but not the spinal cord. In this study, spinal cord astrocytes (SCA) derived from wild type C57BL6J (WT) and Nrf2 knockout (Nrf2 KO) mice were compared for their susceptibility to MeHg toxicity. Examination of viability showed that the Nrf2 KO-derived SCA were more susceptible to MeHg compared to WT-derived SCA. Exposure of SCAs from these two genotypes for 1h to 5µM MeHg, a concentration that was previously reported to decrease SCA viability in WT mice, significantly reduced Nrf2 KO SCA cell viability (60% of the vehicle) when compared to WT SCA. Following vehicle treatment, several genes involving the glutathione synthesis (*Gclc*) and redox status (*Gpx1* and *GPx4*) were reduced in Nrf2 KO SCA compared to WT SCA. Conversely, expression of *Keap1*, *Abcc1* (Mrp1) and *Slc7a11* (system Xc-) mRNA were all significantly higher than in WT SCA. Increase expression of *Abcc1* and *Slc7a11* could reflect compensatory pathway(s) in the maintenance of the redox homeostasis in Nrf2 KO mice. MeHg treatment for 18h reduced the majority of these antioxidant genes in both WT and Nrf2 KO derived SCA. MeHg slightly affected the expression of *Keap1* in WT SCA, but significantly reduced it in Nrf2 KO SCA. After 18h MeHg treatment, *Slc7a11* mRNA was greatly increased in WT SCA (5-fold induction), but it appeared to be reduced in Nrf2 KO SCA. The differential

effects of MeHg on the expression of *Keap1* and *Slc7a*11 in WT and Nrf2 KO are consistent with a gene X environmental effect of MeHg on SCA. *This research was supported by NIH grant R01ES024064, and R25NS090989- ENDURE Program.*

3011 The Impact of Iron Deficiency on Lead Toxicity in the Brain

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Iron deficiency (ID) is the most prevalent micronutrient deficiency in the world. Pregnant women are particularly susceptible due to enhanced iron (Fe) requirements needed for fetal development. Lead (Pb), unlike Fe, is a nonessential divalent metal and still represents a ubiquitous environmental toxin that has been linked to many neurodevelopmental impairments. The neurodevelopmental impacts of ID and Pb exposure have been studied mainly individually with minimal research considering co-exposure. This is surprising considering that ID and Pb both impact areas of lower socioeconomic status and have been associated with similar neurodevelopmental and behavioral deficits. Preliminary data from our laboratory using a dietary model of ID combined with low-level lifelong Pb exposure revealed that offspring of ID rat dams had significantly enhanced brain Pb accumulation compared to iron normal rat dam offspring under identical Pb exposures. Despite previous reports demonstrating positive correlations between brain Pb concentration and behavioral deficits, enhanced Pb accumulation in ID offspring was neither associated with exacerbated functional impairments defined by analysis of the auditory brainstem response (ABR) nor was the behavioral phenotype statistically different compared to that of single-exposed ID or Pb animals. We did however, observe region-specific exacerbated expression of glial fibrillary acidic protein (GFAP), a marker of astrogliosis. Astrocytes may therefore serve a neuroprotective function in the presence of co-exposure contributing to subsequent offspring phenotype. In support of this, preliminary *in vitro* data demonstrated enhanced Pb accumulation in ID astrocytes compared to IN astrocytes using Inductively-Coupled Plasma Mass Spectrometry (ICP-MS). We hypothesize that astrocytic Pb sequestration under ID transiently protects surrounding neurons and oligodendrocytes from Pb-induced toxicity, but the long-term impacts of Pb loading on astrocytes could contribute to neurological consequences later in life. To test our hypothesis, we use *in vivo* and *in vitro* approaches combined with elemental analysis of Fe and Pb using ICP-MS to determine preferential Pb loading into astrocytes and its short- and long-term consequences on astrocytes themselves and survival and function of neurons and oligodendrocytes. *Supported by R01HD094563.*

3012 Regulation of Brain Manganese Homeostasis by SLC30A10

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Manganese (Mn) is an essential metal involved in various biological processes. However, overexposure to Mn is neurotoxic. In humans, loss-of-function mutations in the Mn efflux transporter, SLC30A10, result in an inherited form of Mn toxicity (Tuschl et al., 2012). Patients have increased Mn levels in the brain and liver and present with parkinsonian motor deficits. Mn preferentially builds up in the basal ganglia, specifically in the globus pallidus, and is associated with neuronal death in this region. To gain a better understanding of Mn neurotoxicity and subsequent motor deficits, our lab studies the role of SLC30A10 activity in regulating brain Mn. Our recent work revealed that activity of SLC30A10 in the liver and gastrointestinal tract regulates basal brain Mn levels by mediating Mn excretion. In contrast, activity in the brain reduces brain Mn levels during overexposure (Taylor et al., 2019). Here, we examine the role of SLC30A10 in the brain by using pan-neuronal/glial *Slc30a10* knockout mice, which lack SLC30A10 activity in neurons and glia (astrocytes and oligodendrocytes). Previous results showed Mn-treated knockouts have higher Mn levels in the basal ganglia and thalamus compared to Mn-treated controls. In the present study, we assess motor function of Mn- and vehicle-treated knockouts and controls using the open field test. Mn overexposure alone resulted in reduced locomotion in the open field. Loss of SLC30A10 function in the brain also resulted in reduced locomotion during normal conditions or following Mn overexposure. These results suggest SLC30A10 activity in the brain is critical in protecting against Mn-induced motor deficits. Ongoing work aims to determine the neuronal subtypes most important for the neuroprotective activity of SLC30A10. Results from these experiments provide critical insight into how SLC30A10 activity in the brain regulates brain Mn homeostasis and protects against Mn neurotoxicity and subsequent behavioral deficits.

3013 Lead Poisoning Impairs Thyroid Hormone-Dependent Changes in Brain Development in *Xenopus laevis* Tadpoles

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Lead (Pb) poisoning during early development is associated with behavioral deficits, including low IQ. While the specific mechanisms by which Pb affects brain development are still not fully understood, one hypothesis is that Pb may impair thyroid hormone (TH) signaling by dysregulation of TH distributor proteins (THDPs). To address this issue, we assessed the effects of Pb on TH-dependent cellular and molecular mechanisms of development, with focus on neural development, in *Xenopus laevis* tadpoles (stage 47, ~10 days post fertilization). We found that seven days treatment with Pb decreased expression of two critical THDPs. Pb decreased expression of transthyretin (TTR) in the liver in a dose-dependent manner and 1000 ppb Pb treatment decreased expression of lipocalin-like prostaglandin D_2 synthase (L-PTGDS) in the brain. To determine if Pb impairs TH-dependent mechanisms of development, we treated tadpoles with 1000 ppb Pb bath for 5 days and added one of three different concentrations of thyroxine (T_4) for the final two days of treatment. We found that Pb inhibited the ability of exogenous T_4 administration to initially increase body length compared to controls. Following fixation, we performed immunostaining for the proliferative marker phospho-histone 3 (pH3) on dissected brains and quantified the number of pH3+ cells as well as the volume of the forebrain, midbrain, and hindbrain. We found that Pb exposure impaired T_4-induced increase in neuronal proliferation and impaired T_4-induced changes in brain volume during development. Ongoing experiments will determine whether the effects of Pb on T_4-mediated changes in cell proliferation are likely due to changes in expression of TTR and L-PTGDS in the choroid plexus by using overexpression and knockdown of these genes using transfection of plasmid constructs or morpholinos, respectively. In addition, we are currently examining localization of Pb accumulation in the brain using µCT. The current results from these experiments suggest that Pb impairs TH-dependent mechanisms of brain development, and future results will address the degree to which this is mediated by impairment of THDP function.

3014 Thiol Antioxidant Barrier in Blood Is a Proper Biomarker of Individual Sensitivity for Methylmercury Neurotoxicity in Rats

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The main target organ for methylmercury (MeHg) is the nervous system, and its irreversible neurological dysfunction remains an important issue. Recovering from this dysfunction is very difficult and requires proper biomarkers for individual susceptibility in order to predict and protect against MeHg neurotoxicity. However, a proper biomarker of MeHg neurotoxicity has not yet been established. Previous studies have demonstrated a critical role for oxidative stress in the pathogenesis of MeHg neurotoxicity. Because MeHg has high affinity for selenohydryl groups, sulfhydryl groups, and selenides, proteins with selenohydryl and sulfhydryl groups should play a critical role in mediating MeHg-induced oxidative stress. Plasma oxidative stress markers and selenoproteins were investigated in MeHg-intoxicated rats showing a specific clinical sign (hind limb crossing behavior) and neuropathological changes in cerebellum and spinal peripheral nerve after 4 weeks of MeHg exposure. The thiol antioxidant barrier (-SHp) level significantly decreased 2 weeks after MeHg exposure, which is an early stage at which no systemic oxidative stress, histopathological changes, or clinical sign were detected. In addition, each -SHp level 2 weeks after MeHg administration was significantly correlated with the subsequent neurological symptoms and neuropathological changes. On the other hand, a significant increase in blood oxidation level (d-ROM) and a decrease in selenoprotein P1, which is a selenium-containing protein in plasma, abundance were observed in 4 weeks after MeHg administrated rats. These results suggest that decreased capacity of -SHp can be a proper biomarker of ongoing MeHg neurotoxicity and the individual protective capacity against the MeHg body burden.

3015 Synergistic Toxic Effects and Mechanisms of Lead (Pb) and Disinfection By-Products (DBPs) in Drinking Water on Human Colon Epithelial Caco-2 Cell, Liver HepG2 Cell, and Nervous SH-SY5Y Cell

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Chlorine disinfection aimed at inactivating pathogens is an essential process in drinking water treatment plants. During disinfection, halogenated disinfection byproducts (DBPs) can be generated from the reactions of chlorine with natural organic matter and halide ions in the source water. Halogenated DBPs have been shown to induce chronic adverse effects on cells in culture and rodent models. A certain amount of disinfectant residue is maintained in the water distribution system to prevent the regrowth of microorganisms. Lead (Pb)-containing plumbing materials are widely present in water distribution pipelines in the US Disinfectant residues can react with the pipe materials and enable the release of lead (in the form of Pb^{2+}) into water. Lead is a heavy metal that primarily affects the peripheral and central nervous systems, kidneys, red blood cells, and calcium metabolism after ingestion. In this study, we measured the cytotoxicity of lead (Pb^{2+}) and halogenated DBPs on human cells separately as well as the synergistic cytotoxic effects of Pb^{2+} and DBPs co-exposures, and investigated the mechanisms of ATPase inhibition, ROS generation, calcium dysregulation, and SUMOylation of p53. The human cells selected included the colon epithelial Caco-2 cell, liver HepG2 cell, and nervous SH-SY5Y cell. Data was collected over a dose-response and time-response experimental design and compared to a series of controls. The results showed that the combined toxic effect of lead and each DBP on the cell cultures were both dose and time dependent. Our data suggest that lead affects the formation of DBPs during disinfection (in the water distribution pipelines) and the overall quality of drinking water.

3016 Neuroprotective Effect of N-Acetylcysteine on Mouse Motor-Neuron-Like Cells in Methylmercury-Induced Toxicity

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Methylmercury (MeHg), causes neurodegeneration by mechanisms including oxidative stress and excitotoxicity. MeHg induced overproduction of reactive oxygen species (ROS) is partly due to reduction of glutathione (GSH). Both ROS and MeHg activate the nuclear factor erythroid-derived 2-like 2 (Nrf2) pathway. MeHg also induces glutamate release and causes excitotoxicity. As N-acetylcysteine (NAC) has antioxidant qualities and is a precursor for GSH synthesis, we tested its neuroprotective effects on motor neuron-like cells (NSC34) in MeHg-induced toxicity. Cells were treated for 2h with 0.01, 0.1 or 1mM NAC before exposure to 5µM MeHg. Cell viability was measured every 3h for 36h of MeHg exposure. NAC caused a concentration-dependent protective effect against MeHg-induced cell death. At 1mM, NAC protected NSC34 cells against MeHg for over 36 h. A morphological study using bright filed imaging displayed that 1mM NAC pretreatment or co-treatment with 5µM MeHg for 18h protected NSC34 cells from the loss of synaptic integrity and degeneration. To test whether the mechanism of action of NAC involved the Nrf2 pathway, expression of superoxide dismutase-1 (SOD1) and -2 (SOD2), the downstream paths underlying the Nrf2 pathway were compared to vehicle treatment and MeHg exposure. At 18h MeHg exposure alone, SOD1 and SOD2 expression were up-regulated, which might suggest the cells loss of redox homeostasis, and the Nrf2 antioxidant pathway was activated. NAC co-treatment with MeHg protected the dysregulation of SOD1 and SOD2 expression in these cells. The expression of excitatory amino acid transporter 3 (EAAT3) and subunit 2 (GluA2) of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) were examined to determine if they contributed to dysregulation of glutamate transmission following MeHg exposure. Immunofluorescence results indicated that EAAT3 and the GluA2 subunit appeared to be diminished in MeHg exposure, whereas NSC34 cells treated with 1mM NAC maintained these protein expressions as the same level of vehicle treatments. In conclusion, NAC protected against MeHg-induced neuronal degeneration and its mechanisms of action could contribute to the maintenance of redox and glutamate homeostasis in NSC 34 cells. *This research was supported by NIH grant R01ES024064, and R25NS090989-ENDURE Program.*

3017 Deletion of Astrocytic YY1 Protects against Mn-Induced Dopaminergic Neurotoxicity in Mice

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Chronic overexposure to manganese (Mn) causes a neurological condition, called manganism. Emerging evidence suggests that the transcription factor yin yang 1 (YY1) mediates Mn-induced astrocytic glutamate transporters GLAST/GLT-1 dysregulation, leading to excitotoxicity. In this study, we aimed to test if deletion of YY1 in astrocytes protects mice against Mn neurotoxicity by attenuating Mn-induced repression of GLAST/GLT-1. We injected the adeno-associated viral vector 5 (AAV5) expressing *Cre* recombinase under the control of glial fibrillary acidic protein (GFAP) promoter into the substantia nigra (SN) of YY1-floxed ($YY1^{loxP/loxP}$) mice to generate SN-astrocyte specific YY1 knockout (YY1-cKO). Three weeks after injection, Mn was treated by nostril instillation (30 mg/kg/day, 3 wks), followed by behavioral studies and immunohistochemistry, qPCR and WB analyses. Results showed that YY1 was ablated in SN astrocytes in YY1-cKO mice, displaying normal behaviors. Mn decreased locomotor activity and motor coordination in WT mice, whereas YY1-cKO mice attenuated those Mn-induced deficits. Moreover, at the molecular levels, YY1 deletion attenuated Mn-reduced tyrosine hydroxylase (TH) protein levels, a marker of dopaminergic neurons, in SN and striatum. Mn also decreased GLAST/GLT-1 mRNA and protein levels in SN, whereas deletion of astrocytic YY1 attenuated these Mn effects. These results suggest that astrocytic YY1 plays a critical role in Mn-induced dopaminergic toxicity, possibly via dysregulation of astrocytic GLAST/GLT-1, leading to excitotoxic neuronal injury. These findings indicate that astrocytic YY1 may serve as a potential target for therapeutics to treat Mn toxicity. *Supported by National Institutes of Health, National Institute of Environmental Health Sciences (NIH/NIEHS): R01 ES024756 (EL), R01 ES10563 (MA), R01 ES10563 (MA), R01 ES07331 (MA) and 1R21 ES025415 (MA).*

3018 Chronic Early-Life Lead Exposure Disrupts Behavior and µ-Opioid Receptor Levels in the Rat Brain: Implications to Mental Disorders

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Environmental factors have been associated with psychiatric disorders and recent epidemiological studies suggest an association between prenatal lead (Pb^{2+}) exposure and schizophrenia (SZ). Preclinical studies show that developmental Pb^{2+} exposure recapitulates specific neuropathological and dopaminergic system changes present in SZ. However, the mechanisms underlying the negative symptoms of SZ have not been fully elucidated. µ-opioid receptors (MOR) are key in hedonic functions and reward pathways, and have been hypothesized to play a role in the negative symptomatology of SZ, as MOR levels are decreased in SZ patients (Ashok et al, 2019) In this study, we examine the effect of chronic early life lead exposure (CELLE) on sensorimotor gating using pre-pulse inhibition of the acoustic startle (PPI) and MOR levels in the brain of rats in early adolescence (postnatal day 28, PN28), late adolescence (PN50) or adulthood (PN120). Our CELLE paradigm results in blood lead levels averaging ≤1.9 (control) or 22 ± 0.07 µg/dL (Pb^{2+}) at PN28. Chronic Pb^{2+} exposure did not affect PPI in PN28 male rats ($F_{1,35}$ = 0.54, p=0.47), however PPI deficits were present at PN50 and PN120 ($F_{1,35}$= 11.3, p=0.002, $F_{1,35}$= 9.3, p=0.004 respectively), consistent with the age trajectory of PPI deficits in SZ. We found no effect of Pb^{2+} exposure on PPI in female rats. MOR levels in relevant brain regions were measured using autoradiography. At PN28 MOR levels were increased in the striatum (p=0.07), nucleus accumbens (NAC), p=0.04; basolateral amygdala (BLA), p=0.002; and thalamic nuclei (lateral post-thalamic nuclei (LPTN), p=0.003; stria medullaris of the thalamus, p=0.01) of male Pb^{2+} rats compared to controls. PN28 Pb^{2+}females had increased levels of MOR in NAC, p=0.005; BLA p=0.04, LPTN p=0.006 and medial thalamus p=0.002. No changes in MOR levels were observed at PN50 in both sexes, and there was a decrease in MOR levels in medial thalamus (p=0.02) of Pb^{2+} exposed males at PN120 compared to controls. No changes were observed in females at PN120. These studies show that chronic early life Pb^{2+}-exposure results in PPI inhibition, an endophenotype for SZ and produces differential changes in the opioid system. Our findings along with previous studies suggest the role of environmental factors, such as Pb^{2+}, as a risk factor for mental disorders and indicate that our CELLE model may serve as an environmental animal model of SZ. *NIEHS grant #ES006189-25 to TRG.*

3019 Mechanism of Mn-Induced YY1 Activation via Aurora B Kinase and Casein Kinase II in Astrocytes

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Chronic exposure to manganese (Mn) causes a neurological disorder known as manganism. The mechanisms underlying Mn neurotoxicity are not well understood, but includes dysregulation of the astrocytic glutamate transporter excitatory amino acid transporter 2 (EAAT2), leading to excess extracellular glutamate levels and subsequent excitotoxicity. Decreases in EAAT2 expression and activity are associated with pathogenesis in several neurological disorders, including Parkinson's disease (PD) and manganism, and involve the repressive action of the transcription factor Yin Yang 1 (YY1). In the present study, we investigated the mechanisms of Mn-induced YY1 activation to determine if Mn increases YY1 expression via the cytokine TNF-α, oxidative stress and activation of NF-κB signaling. We also tested if Mn activates YY1 via phosphorylation, leading to the transcriptional repression of EAAT2. Our results demonstrate that Mn exposure induces phosphorylation of YY1 via kinases Aurora B kinase (AurkB) and Casein kinase II (CKII), leading to increases in YY1 nuclear translocation, YY1 interaction with histone deacetylases (HDACs 1 & 3), and EAAT2 repression. Mn-induced TNF-α production and oxidative stress was also associated with proinflammatory signaling upstream of NF-κB. Accordingly, treatment with IKK16, an inhibitor of IKKβ, and antioxidants attenuated Mn-induced activation of NF-κB and YY1 and restored EAAT2 expression. Taken together, these results indicate that Mn-induced oxidative stress/inflammation upregulates YY1 via NF-κB, and that Mn also induces YY1 phosphorylation, leading to EAAT2 reduction and subsequent excitotoxic neuronal injury.

3020 Role of APL-1 in Manganese- and Paraquat-Induced Toxicity in *Caenorhabditis elegans*

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Alzheimer's disease affects over five million Americans and is the sixth leading cause of death. The molecular mechanisms and factors that trigger the onset and disease progression remain largely unknown. However, misfolding and aggregation of β-amyloid (Aβ) plaques in the brain characterize the disease secondary to changes in amyloid precursor protein (APP) processing. Environmental and occupational factors such as manganese (Mn) and paraquat (PQ) have been implicated in the etiology of Alzheimer's disease and the ensuing changes in APP cleavage. Here we used *Caenorhabditis elegans* (*C. elegans*) as a model to explore putative mechanisms of neurodegeneration secondary to exposure to Mn or PQ. Specifically, APL-1, the *C. elegans* orthologue of mammalian APP was studied to evaluate its role in the disease. Studies were carried out in wild-type N2 and APL-1 (yn5) strains to assess sensitivity to reactive oxygen species (ROS) generation, as well as in BY200 worms, where dopaminergic neurons are labeled with green fluorescent protein (GFP) allowing for the evaluation of neurodegeneration. The results showed that the APL-1 strain was more sensitive to Mn or PQ than wild-type worms. Moreover, we observed increased levels of ROS upon exposure to Mn (25mM) and PQ (0.5 and 1mM) in N2 worms compared with controls. Worms exposed to Mn or PQ showed increased dopaminergic neurodegeneration, consistent with the increased ROS levels. Our results suggest that both Mn and PQ cause increase in ROS levels and neurodegeneration.

3021 Essential Amino Acids Are Crucial for Manganese Potentiation of Insulin/IGF Dependent S6, but Not AKT Phosphorylation

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Manganese (Mn) is essential for life, including brain development, nerve and cognitive functioning. Several kinases of the insulin/IGF signaling cascade are Mn-dependent. Studies have shown that Mn activates kinases in the IGF pathway including AKT, mTOR which are neuroprotective in Huntington's disease. As Mn is a known insulin mimetic, the overarching goal here was to identify direct molecular targets in insulin signaling pathway that are altered at non-cytotoxic threshold exposures. Prior *in vitro* studies have established that Mn-dependent AKT signaling is synergistically augmented by IGF (1nM) and Mn at higher concentrations (200 and 500 μM) acting at insulin/IGF receptors (IR/IGFR). AKT (S473) phosphorylation consistently occurs under simplified saline buffer exposures, but the response of the ribosomal protein kinase S6 (S235/236), downstream of AKT and mTOR in the IGF signaling cascade has shown inconsistent effects. We hypothesize that essential amino acids (EAA) play a role in Mn dependent activation of S6 kinases. To test this hypothesis, we subjected an *in vitro* model, *STHdh*, murine striatal cell lines to insulin/IGF deprivation and followed by a 3 hour exposure to Mn (100 μM), and/or IGF (1 nM) and IR/IGFR inhibitor (BMS-536924 at 100 nM) in both the presence and absence of EAA. We observed that in complete media with EAA and insulin, AKT phosphorylation increased at 200 μM Mn but not at 100 μM Mn. Mn mediated increases in AKT phosphorylation were seen in both the presence and absence of EAA which was inhibited by BMS. Furthermore, in support of our hypothesis, we observed a Mn potentiated increase in S6 phosphorylation occurred exclusively in the presence of EAA, and BMS blocked this effect. We find that IGF alone does not increase the phosphorylation of S6 in absence of EAA, despite AKT activation. In addition, 100 μM Mn potentiates the effect of IGF on S6 only, not AKT, in a Mn-dependent manner through IGF signaling pathway only in the presence of EAA. This suggests a potential parallel pathway through which EAA is facilitating the Mn- dependent phosphorylation of S6. We are exploring the effects of Mn on insulin/IGF signaling using a similar exposure paradigm in other model systems. This study will help us better understand the effects of Mn on insulin signaling pathway, which is crucial in context Mn neurotoxicity.

3022 Determination of *In Vitro* Methylmercury Exposure Paradigms Relevant to *In Vivo* MeHg Levels in the Human Developing CNS

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The human developing brain shows high vulnerability to methylmercury (MeHg) and the severity of the symptoms seem to depend on exposure levels, duration and developmental stage. To assess the effect of MeHg on the developing CNS, we established hiPSC-derived neuronal cultures. We argue that physiologically relevant *in vitro* toxicant exposure paradigms are best determined by comparing toxicant levels accumulated in *in vitro* cells with levels measured *in vivo*, rather than translating *in vivo* levels into cell culture medium concentrations. Here, we set out to determine relevant MeHg exposure paradigms to study the effect of MeHg on developing human neurons. Developing human mesencephalic dopaminergic floor plate cells differentiated from hiPSC were exposed to 100 nM or 1 μM MeHg continuously for 5 days during the proliferative neural precursor cell (NPC) stage. After the end of the 5 day exposure cell samples were collected daily and MeHg content measured and normalized to protein content. 24 hours after the last exposure cellular Hg contents were 3.3 ± 0.08 ng/mg protein and 37.8 ± 2.70 ng/mg protein for the 5 day long exposures to 0.1 μM and 1 μM, respectively. Based on our calculations using published values for brain density (1.05 g/ml) and brain protein concentration (100 mg protein/g wet weight) we determined that a 5 day exposure to 1 μM MeHg results in a cellular Hg content within the range that has been reported to cause delayed psychomotor development (3 ppm), while the same exposure paradigm at 0.1 μM MeHg resulted in levels just below the reported lowest-observed-adverse-effect-level (0.5-1 ppm). We further found that dopaminergic NPCs extrude about 60% of the accumulated Hg every 24 hours for the first 48 hours with a decreasing rate thereafter. Neither of these two exposure paradigms (100 nM and 1 μM for 5 days) resulted in loss of cell viability or changes in proliferation rates. We are currently testing if Hg accumulation and extrusion differs during different neuronal differentiation stages and in different neural lineages and are determining if there are any MeHg-induced effects on functional phenotypes including neuronal electrical activity. *NIEHS R01 ES007331, ABB, MA.*

3023 Developmental MeHg Exposure Disrupts Stage-Specific Neuronal Differentiation in a Human-Induced Pluripotent Stem Cell Model

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Although methylmercury (MeHg) is an extensively studied developmental neurotoxicant, little is known about which neuronal lineages and stages of development are most sensitive to MeHg exposure. Exposures in both mammalian and invertebrate models have suggested that MeHg may target dopaminergic neurons. However, historic accidental poisonings of MeHg have

shown that loss of cerebral and cerebellar glutamatergic neurons is a hallmark of MeHg toxicity. Additionally, it is unknown whether this loss was due to MeHg inhibiting differentiation, migration, or to neuronal death. Therefore, we aimed to understand which neuronal lineages and stages of development are most sensitive to MeHg exposure, using the human-induced pluripotent stem cell (hiPSC) model. hiPSCs were differentiated down the cortical glutamatergic and the floor plate dopaminergic lineages, and exposed to 0, 0.1, or 1μM MeHg. Exposures occurred five days over the course of development to the neuroprogenitor (NP) stage (days 4 to 10 of differentiation) and/or five days over the course of development of early post-mitotic neurons (days 14 to 20 of differentiation). Cell lysates were collected for protein quantification and total mercury quantification. Differentiation potential was determined by examining expression of lineage-specific markers with qRT-PCR at days 11 and 21 of differentiation. In the cortical lineage, MeHg exposure did not significantly affect expression of NP markers at day 11. However, at day 21, MeHg was shown to cause an increase in expression of FOXG1 (n=3, one-way ANOVA, p=0.0384), a telencephalic NP marker, and a corresponding decrease in expression of TBR1 (n=3, one-way ANOVA, p=0.0271), an early deep layer cortical glutamatergic neuron marker. In concordance with these results, we saw a continued mercury bioaccumulation over the 14 to 20 day exposure window. In contrast, during the day 4 to 10 exposure window, mercury concentration appeared to reach saturation after the first 24 hours of exposure. This suggests differences in transport or storage of MeHg between these stages of development may play a role in the sensitivity of differentiating NPs to MeHg. Future studies will focus on the floor plate dopaminergic lineage and the mechanisms by which MeHg disrupts neuronal development in a stage- and lineage-dependent manner.

3024 Molecular Mechanisms of Cobalt-Induced Toxicity in *Caenorhabditis elegans*

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As a component of vitamin B12, cobalt is an essential metal. However, high levels of cobalt may lead to metallosis, characterized by sensorineural hearing loss, visual and cognitive impairment and peripheral neuropathy. Excessive serum cobalt can arise from a variety of sources, including diet, exposure from industrial fabrication, or from alloys used in tooth and hip joint replacements. In a long-term follow-up human trial, the mean serum cobalt concentration was 4-fold higher in the cobalt chrome metal-on-metal arthroplasties (1.5 μg/L) compared with the metal- on-polyethene cohort (0.4 μg/L, P < .001). Previously, we have shown that cobalt overexposure results in defects in rat neural cells, including lethality, hypoxia and the loss of learning, memory and spatial-exploration abilities. In the present study, we sought to unveil the molecular mechanisms of cobalt induced neurotoxicity in *Caenorhabditis elegans* (*C. elegans*). Exposure to cobalt chloride for 2 h significantly decreased the survival rates in the nematodes (IC50= 20.69 mM). As a common consequence of cobalt exposure, oxidative stress was detected using 2′,7′-dichlorodihydrofluorescein diacetate / 2′,7′-dichlorofluorescein (H2DCFDA) assay and a reporter strain VP596 (reporter gene construct). In VP596 animals, GFP (green fluorescent protein) is driven by the promoter of glutathione S-transferase 4 (*gst-4*) gene which is activated upon oxidative stress. As an upstream regulator of *gst-4*, and a homologue of the nuclear factor erythroid 2-related factor 2 (Nrf2) in mammals, *skn-1* levels were found to be significantly upregulated by cobalt induced oxidative stress. Furthermore, we identified mitochondrial oxidative stress by MitoTracker assay and altered mitochondrial morphology by confocal microscopy following cobalt exposure. mRNA levels of mitochondrial dynamic related genes such as *drp-1*, *fzo-1* and *miro-1* have also changed accordingly. In addition, qRT-PCR results indicated that genes regulating apoptosis (*egl-1*, *ced-3*, *ced-4*, *ced-9*) and autophagy (*bec-1*, *lgg-1*) were altered in cobalt induced toxicity. In conclusion, our results demonstrate that cobalt exposure can induce oxidative stress and mitochondrial dysfunction, in turn, leading to lethality, activation of autophagy and induction of apoptosis in *C. elegans*. *The study was supported by NIEHS (R01ES07331, R01ES10563), NSFC (81903352) and Provincial Natural Science Foundation of Fujian Province (2019J05081).*

3025 Chronic Exposure to Methylmercury Induces Puncta Formation in CEP Dopaminergic Neurons in *Caenorhabditis elegans*

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The neurotoxin methylmercury (MeHg) produces dopaminergic neuron damage in various models. Previous studies have demonstrated the utility of *Caenorhabditis elegans* (*C. elegans*) as an alternative and complementary experimental model in dissecting out mechanism of MeHg-induced dopaminergic neurodegeneration. However, sensitive morphological biomarkers of dopaminergic neurodegeneration that precede frank MeHg neurotoxicity and neuronal cell death are lacking. By establishing a chronic exposure *C. elegans* model using OH7193 strain, we have discovered a propensity of MeHg exposures (5 μM, 10 days) to induce bright puncta of *dat-1*::mCherry aggregates(>70%) specifically in the dendrites of CEP dopaminergic neurons in a dose- and time-dependent manner. In contrast, the puncta formation was not induced in other neurons including: 2 ADEs, 2 PDEs, cholinergic neurons (2 AIYs) or glutamatergic neurons (2 PVDs). Features of the puncta, along with shrinking dendrites are distinct from those inherent to normal mCherry fluorescent protein aggregates, and instead resemble the dendritic varicosities in substantia nigra (SNc) neurons of patients with Parkinson's disease (PD). Moreover, the expression levels of *gst-4* and tubulins (*tba-1* and *tba-2*) genes were not significantly modified under this chronic exposure paradigm, though *gst-4* showed significant changes in a one day exposure paradigm. We hypothesize that the puncta formation occurs from a diverse set of mCherry-linked cellular macromolecules altered by MeHg, which could exhibit a localized, high intensity of mCherry signal. We are continuing to investigate the components of the puncta using this model.

3026 Manganese-Induced ROS and AChE Variants Alteration Leads to SN56 Basal Forebrain Cholinergic Neuronal Loss after Acute and Long-Term Treatment

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Manganese (Mn) induces cognitive disorders and basal forebrain (BF) cholinergic neuronal loss, involved on learning and memory regulation, which could be the cause of such cognitive disorders. However, the mechanisms through which it induces these effects are unknown. Mn induces oxidative stress generation, and alters AChE gene expression in other brain regions, effect involved with BF cholinergic neuronal loss. Otherwise, cholinergic system, mainly in BF, is involved on learning and memory regulation, and an alteration of cholinergic transmission in BF, as Mn produces in other brain regions, could induce these effects. Thus, we hypothesized that Mn could induce BF cholinergic neuronal loss through oxidative stress generation, cholinergic transmission and AChE variants alteration. This study shows that Mn impaired cholinergic transmission in SN56 cholinergic neurons from BF through alteration of AChE and ChAT activity and CHT expression. Moreover, Mn induces, after acute and long-term exposure, cell death on SN56 cholinergic neurons and this effect is independent of cholinergic transmission alteration, but was mediated partially by oxidative stress generation and AChE variants alteration. Our results provide new understanding of the mechanisms contributing to the harmful effects of Mn on cholinergic neurons and their possible involvement in cognitive disorders induced by Mn.

3027 Sex-Specific Effects of Developmental Dieldrin Exposure on Susceptibility to α-Synuclein Preformed Fibrils in Mice

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Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neurons in the nigrostriatal pathway and the formation of α-synuclein-containing Lewy bodies. While an abundance of research has investigated specific PD-linked genetic loci, the majority of PD cases (~90%) are sporadic (i.e. not caused by monogenically inherited *mutations*) and are likely due to a complex interaction between genes and environmental factors. Building on this concept, epidemiological and mechanistic studies have shown an association between exposure to persistent organic pollutants, including pesticides and industrial toxicants, and an increased risk of PD. One potential mediator of the relationship between environmental exposures and PD is the epigenome, which is sensitive to the environment, established during cellular differentiation, and regulates gene expression throughout the lifespan. Our research aims to determine whether developmental exposures, in particular to the organochlorine pesticide dieldrin, alters PD risk by establishing a poised epigenetic state early in life that mediates susceptibility to parkinsonian neurotoxicity in adulthood. To test this hypothesis, we combined a developmental dieldrin exposure paradigm with the α-synuclein (α-syn) pre-formed fibril (PFF) model in mice. In a previous study, we found that dieldrin exposure establishes a sex-specific poised epigenetic state early in life, including at genes that may mediate susceptibility to neurotoxicity into adulthood. Here, we showed sex-specific effects of developmental dieldrin exposure on expression of genes involved in neuroinflammation. We also observed a male-specific exacerbation of PFF-induced deficits in dopamine packaging and motor behavior by dieldrin exposure in the challenging beam test six months after PFF injections. In contrast, female mice did not show a dieldrin-induced exacerbation of PFF-induced toxicity, and they did not show any PFF-induced motor deficits on the challenging beam test despite similar PFF-induced neuropathological and neurochemical changes. The dieldrin-induced, male-specific increase in neuronal vulnerability is consistent with previous results in the MPTP model, and reflects the increased prevalence of PD in males. Taken together, our results suggest that the exposure+PFF two-hit model is a novel paradigm that can be used to explore the mechanisms by which PD-related exposures alter neuronal vulnerability to synucleinopathy in Parkinson's disease.

3028 Neurological Effects of *In Vivo* Chlorpyrifos Exposures in Rats

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Military personnel are at risk for exposures to various chemicals such as pesticides, which are common contaminants found in the environment, food, and water. Adverse neurological effects of pesticide exposures have been documented in Gulf War veterans. The objective of this project is to characterize the neurological effects of *in vivo* chlorpyrifos (CPF) exposures on neurological function, using not only behavioral testing but also electrophysiology to directly assess neuronal function. A secondary objective of this project is to explore whether administration of transcranial direct current stimulation (tDCS) can alleviate the detected neurological effects. Rats were exposed to a single dose of CPF via oral gavage and endpoints were determined approximately 3 hours post exposure to assess acute effects and >50 days post exposure to assess delayed effects. Dose levels were 0 (control), 10 (low), 30 (mid), or 60 (high) mg/kg body weight. We found statistically significant reduction in motor activity of animals 3 hours following a one-time gavage of 60 mg/kg CPF (high). There was an increased startle response in animals exposed to the high dose of CPF. Electrophysiology data revealed statistically significant reduction in paired pulse facilitation (PPF), a measure of short term synaptic plasticity, in hippocampal neurons. These effects were transient and no longer observed 50 days post exposure. No additional delayed effects were found. We also determined that tDCS administration in high dosed CPF group did not significantly alter the reduced motor activity observed at 3 hours post exposure. We also assessed the effects of repeated CPF exposures. Here, animals were exposed to 0 (control), 5 mg/kg (mid) or 10 mg/kg (high) CPF for 5 days/week over a 2-week period. Data analyses are underway and our results thus far revealed statistically significant reduction in motor activity at 1 day following the last day of exposure in animals that were repeatedly exposed to CPF. In conclusion, we found that exposure to CPF induced a significant but transient reduction in motor activity as well as decreased PPF in the hippocampus. Furthermore, tDCS was unable to reverse the reduced motor activity of animals that were singly exposed to CPF.

3029 Human Allele-Specific Methylated Regions Have Increased Resilience to Rotenone Exposure in α-Synuclein Knockdown Neurons

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Allele specific DNA methylation (ASM) describes genomic loci that maintain CpG methylation at only one inherited allele rather than having coordinated methylation across both alleles. The most prominent of these regions are ASMs that control the expression of imprinted genes and are commonly associated with genetic disorders such as Beckwith-Wiedemann and Prader-Willi syndromes. Imprinted genes are significantly enriched in the brain and their regulation influences cognitive function and behavior throughout aging. They are dependent on DNA methyltransferase I (DNMT1) and are hypothesized to be critical for mechanistic insights into environmental disease. We have identified DNMT1 dependent regions in the human genome which include both germline ASMs and non-traditional ASMs. We explored the vulnerability of these regions to environmental factors using a rotenone induced neuronal cell model. We observed significant up-regulated expression (>1.5 fold change) and hypomethylation at two genes, *HCN2* and *NEFM*, with implications in neurodegeneration. Intriguingly, α-Synuclein had an unexpected role in connecting ASMs to exposure sensitivity. α-Syn is a protein expressed in neurons to support synaptic transmission but misfolded α-Syn can promote the formation of Lewy bodies, a pathological hallmark in neurodegeneration. We have shown increased resilience of SH-5YSY α-Syn knockdown (KD) cells to mitochondrial damage induced by rotenone. We analyzed the transcriptome of rotenone treated wild-type and α-Syn KD SH-5YSY with RNA sequencing. Rotenone altered the expression of 526 genes in wild-type and 128 genes in α-Syn KD cells (1.5 fold change; FDR<0.05). In rotenone treated α-Syn KD, the cellular defense response was significantly enriched ($p<10^{-3}$) and three DNMT1 dependent imprinted genes were significantly up-regulated compared to wild-type. We are currently investigating changes in DNA methylation at these imprinted regions as well as in other predicted ASMs to determine if targeting α-Syn promotes cellular resilience to rotenone by maintaining DNA methylation patterns. This work will provide additional mechanistic insights into gene environment interactions in neurodegeneration and provide support to the hypothesis of developmental origins of adult disease.

3030 The Chronic Exposure to Herbicide Atrazine Increases the Expression of Genes Associated to GABA and Glutamate Systems in Albine Male Rats

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The herbicide atrazine (ATR; 2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is widely used to destroy grasses and broadleaf weeds in crops like corn, sorghum and some fruits. Several studies in rodents have shown that acute, repeated or chronic exposure to ATR is associated with alterations in the nigrostriatal dopaminergic pathway, while its effects on GABAergic and glutamatergic (Glu) pathways have been barely studied. For the evaluation of the ATR effects on GABAergic and glutamatergic systems, Sprague-Dawley male rats were exposed daily to 1 or 10 mg ATR/kg of BW for 12 months. At the end of the ATR treatment, the levels of mRNA of several genes involved in the production, vesiculation, reuptake, and receptors of GABA and Glu in striatum (str), nucleus accumbens (NAcc), prefrontal cortex (PFC), ventral midbrain (vMID) and hippocampus (HIPP) were evaluated by absolute qPCR. Increases in the expression of the glutaminases *gls* and *gls2* in STR, NAcc, HIPP and PFC of rats exposed to 1 and/or 10 mg ATR were found. For the GABAergic genes, increased expression of *gad67* and *vgat* in STR and/or vMID in rats exposed to 1 and/or 10 mg ATR were detected. The expression of genes involved in the glutamatergic system, like *vglut-2* and *grin1* in HIPP of rats exposed to 1 and/or 10 mg ATR we also increased, as was *gria1* in STR and PFC in the group exposed to 1 mg ATR. In the same fashion, the expression of *glast* and *mglur1* increased in STR of rats exposed to 1 and 10 mg ATR groups. These findings show that the GABAergic and Glu systems are targets of ATR exposure. *Funding: UNAM-DGAPA-PAPIIT IN203916, IN208119, and CONACYT 251510.*

3031 Acute Neurotoxicologic Evaluation of Sabadilla Alkaloids

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Sabadilla, *Schoenocaulon officinale* Grey (Liliaceae), has long been used in folk medicine as a botanical species with insecticidal potency and low mammalian toxicity. Among the alkaloid fraction of the seeds (3-6%) which makes up the insecticidal activity, two lipophilic alkaloids, veratridine and cevadine, have the highest reported insecticidal potency. Sabadilla alkaloids, like pyrethrins, exert neurotoxic activity in insects by inhibiting the closing of Na+ channels and disrupting cell membrane depolarization. The potential of SABA-10 (formulation consisting of 10% Sabadilla alkaloids) to produce adverse effects was investigated using an acute oral neurotoxicity study in rats. Test substance-related observations in female rats included abnormal gait, ataxia, hypoactivity, hyperactivity, immobile, irregular respiration, hunched posture, and tremors. Additionally, significant increases observed in female foot splay and motor activity on Day 1 were indicative of transient neurobehavioral impairment. Functional observations that were considered relevant indications of short term adverse detailed clinical/functional signs in female rats included inactivity, ataxic/impaired gait, incapacitation, impaired locomotion, tremors, unusual behaviors (i.e. head shaking, opening and closing of mouth), and slow surface righting. The NOAEL for acute neurotoxicity of SABA-10 was determined to be 50 mg/kg/day for female and 400 mg/kg/day (the highest dose tested) for male rats. All adverse clinical and functional observations on the day of dosing resolved within 24 hours or by the next interval, with no histopathological evidence of permanent central or peripheral nervous system findings.

3032 Identifying the Genetic Determinants of Physiological Response to Combinatorial Environmental Exposures

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C. elegans behavior is conditioned by the cumulative interaction of many factors, such as natural environmental stimuli and manufactured toxicants (Anderson et al., 2004). Specifically, we are interested in how deleterious environmental exposures, alone or in combination, can modulate behaviors mediated by serotonin, an important neurotransmitter conserved across nearly all animal phyla. We focus on two exposures that are known to affect serotonin-dependent behaviors: (1) high temperature as a model for warmer climate and (2) the common pesticide chlorpyrifos. We aim to use genetic methods to identify genetic loci that convey resistance of the serotonergic system when under environmental stress by either temperature, chlorpyrifos, or both. We hypothesize that specific gene networks control the function and resilience of the nervous system, and that combinatorial exposures may uncover new nodes of stress response and resilience. For this, we will use of a resource available in the common genetic model system *C. elegans* where several, genetically-distinct wild isolates are available using a natural diversity resource, (Cook et al., 2016). This will address a key gap, as behavior has been assessed only in the wild type isolated from Bristol, England. By using a model for serotonin-mediated behaviors developed by our lab and an added heat stimulus model, the behavioral profile of each wild isolate will be investigated across temperature ranges and chlorpyrifos concentrations to assess which wild type isolates are able to convey enhanced thermotolerance through survivability. Preliminary data from the seven strains which are from diverse regions, including the Bristol, England strain as a control were compared at several temperatures, focusing on the critical temperature of 33°C for percent of worms surviving (40 worms per replicate, 1 trial each). Fold change for strain with the origin of Israel, between control was 13.84% and for strain with the origin of Peru was 4.33%, indicating enhanced survivability. When survival and behavior have been measured, we will use Quantitative Trait Loci (QTL) analyses to identify the loci responsible for increased or decreased sensitivity to these exposures alone or in combination. We therefore hypothesize that this synergistic exposure will adversely impact the serotonergic system and conveyed thermotolerance, evident in altered serotonin-mediated behaviors. Genetic analysis of specific gene networks will undercover how animals from different environmental conditions and that are exposed to varied environmental stimuli can differ in terms of behavior, and what key genetic differences can contribute to this.

3033 *CHD8* Expression Influences Pesticide-Induced Neurotoxicity in a Mouse Model of Autism

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The considerable plasticity of the developing brain renders it exceptionally vulnerable to genetic and environmental perturbations. Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder with a strong but complex genetic component associated with key molecular pathways early in development. Yet, genetic risk factors seem insufficient to explain an increase in ASD prevalence over the past 15 years, raising the possibility that nonheritable risk factors are also at play. Exposure to exogenous agents during a critical developmental period has been suggested to contribute to ASD etiology. However, given the evidence on ASD heritability, environmental factors that play a role in ASD development likely influence mechanisms also involving some element of genetic susceptibility. Thus, there is an urgent need to identify mechanisms by which nonheritable factors may interact with susceptibility genes. In this study we investigate how haploinsufficiency in one of the most high confidence ASD risk genes, Chromatin Helicase DNA Binding Protein 8 (*CHD8*), influences pesticide-induced neurotoxicity. *In vitro* assessment of neuronal activity, as measured by expression of immediate early genes, c-fos and Npas4, suggests that *CHD8* influences neuronal excitability upon exposure to deltamethrin, an insecticide that functions by inhibiting sodium channel function. RNA-sequencing of cortical tissue at postnatal day 5 following *in vivo* developmental exposure describes an impairment in pathways involved in the maintenance of excitatory-inhibitory balance in *CHD8* mutant mice that is further enhanced by deltamethrin exposure. Developmental exposure to deltamethrin was also associated with autism-like phenotypes at 6 months of age, suggesting that inappropriate neuronal excitability during development can result in behavioral abnormalities later in life. Altogether, these data suggest that homeostatic mechanisms expressed to maintain appropriate levels of neuronal activity despite ongoing challenges to the network are altered in *CHD8* mutant mice influencing the development of autism-like behaviors. Our results generate new knowledge on the possible mechanisms underlying one of the most common autism-associate*d mutations* and contributes to the identification of environmental risk factors for ASD.

3034 Mitochondrial Stress Modulates Elongator Protein 3 (ELP3) Function and Subcellular Localization in Cell Culture and Animal Models of Parkinson's Disease

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Parkinson's disease (PD) has increasingly been linked with mitochondrial dysfunction. Exposure to mitochondria-targeting neurotoxicants, e.g., those neurotoxic pesticides that act as mitochondrial complex I inhibitors, has been linked to the pathogenesis of environmentally linked PD, but the molecular mechanisms underlying the enhanced vulnerability of dopaminergic neurons to mitochondrial toxicants are not fully understood. The histone acetyltransferase ELP3 (Elongator Protein 3) is the catalytic subunit of the RNA polymerase II elongator complex and plays an essential role in transcript elongation and maturation of projection neurons. Dysregulation of ELP3 has been implicated in a number of human disorders, but its potential role in the pathogenesis of PD remains elusive. In this study, we observed that the mitochondria-targeting pesticides rotenone and tebufenpyrad can disrupt ELP3's function in dopaminergic neurons in cell culture and rodent models of PD. Particularly, treatment with mitochondria-targeting pesticides upregulated ELP3 at both the mRNA and protein levels. In vehicle-treated dopaminergic neurons, ELP3 was present in the nucleus, mitochondria and cytosol. However, in neurons exposed to mitochondria-targeting pesticides, ELP3 levels were significantly decreased in both the nucleus and mitochondria but increased in the cytosol. These results suggest that mitochondria-targeting pesticides induce translocation of nuclear and mitochondrial ELP3 to the cytoplasm. Overall, our results suggest that ELP3 is a novel cellular target in models of PD that undergoes characteristic changes in expression levels and subcellular localization in dopaminergic neurons in response to neurotoxic, mitochondria-targeting stressors. *NIH grants ES026892 and ES027245 and Eugene and Linda Lloyd Endowed Chair.*

3035 The Repeated Injection of the Insecticide Thiacloprid Alters Striatal Dopamine Levels in the Albino Male Rat

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Neonicotinoid insecticides are used in agriculture and for the treatment of ectoparasites in domestic pets. These pesticides have been associated with neurotoxic effects in the dopaminergic systems since they can increase the striatal DA release and increase the tyrosine hydroxylase immunoreactivity in substantia nigra pars compacta, in addition to causing hyperactivity in rats. The neonicotinoid insecticide thiacloprid (THI) is used for the treatment of potato, oilseed monkfish, pome fruit, vegetables, and ornamental crops and its use worldwide has gradually increased since 2005. In order to evaluate the effects of the repeated exposure to THI, male rats received an IP injection with 40 mg THI/kg of BW three times a week for two weeks while control group received sterile bi-distilled water injections. Fifteen minutes before and 2 hours after injection, locomotor activity was recorded. Forty-eight hours after the last THI injection striatum (STR), nucleus accumbens (NAcc) and ventral midbrain (vMID) were collected for DA and metabolites analysis. We found alterations in locomotor activity after THI administration. The striatal levels of dopamine were decreased without alterations in its metabolites. These data point to the nigrostriatal system as a target of the neonicotinoid THI. More studies are necessary in order to unveil the specific effects of THI in the nigrostriatal dopaminergic system. *Funding: UNAM-DGAPA-PAPIIT IN208119 and CONACYT 251510.*

3036 Salubrinal Protects against Deltamethrin-Induced Disruption of Hippocampal Neurogenesis in Mice

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Environmental factors, including pesticide exposure, are thought to be significant contributors to the neurodegeneration and cognitive deficits. Emerging evidence indicates that adult neurogenesis in the hippocampus is important for learning and memory. Approximately 1400 newborn neurons are added to adult human hippocampus every day and provide about 2% annual turnover during aging, suggesting that adult neurogenesis contributes significantly to human hippocampal function (Spalding et al., 2013). In our previous study, repeated exposure to deltamethrin (3 mg/kg) caused ER-stress, reduction of hippocampal neurogenesis, and deficits in learning and memory in adult mice. Here, we sought to determine the potential relationship between ER stress and hippocampal neurogenesis following exposure to deltamethrin. Immunohistochemistry of various markers along with bromodeoxyuridine (BrdU) were used to examine neurogenesis. We found that single oral exposure to deltamethrin (3 mg/kg) resulted in reduction of progenitor cell proliferation as BrdU- and Ki67-positive cells were significantly decreased in the dentate gyrus (DG) of the hippocampus. Furthermore, deltamethrin-treated mice exhibited significant reduction in the expression of doublecortin (DCX)-positive cells, suggesting potential impairment of hippocampal neurogenesis. These were accompanied by ER stress as the protein levels of CHOP and GRP78 were significantly increased in the hippocampus following exposure to deltamethrin. To determine whether ER stress is associated with inhibition of neurogenesis, a group of mice were treated with two intraperitoneal (i.p.) injections of 1 mg/kg salubrinal (eIF2α inhibitor) 24 h and 30 min before the administration of deltamethrin. The data revealed that salubrinal prevented ER stress and attenuated deltamethrin-induced reduction of BrdU-, Ki67-, and DCX-positive cells in the DG of hippocampus. Collectively, these results demonstrate that exposure to deltamethrin leads to ER stress mediated inhibition of adult hippocampal neurogenesis, which may subsequently contribute to deficits in learning and memory in mice. *Supported in part by R01ES027481.*

3037 Disease-Toxicant Screen Reveals a Neuroprotective Interaction between Mutations in Alpha-Synuclein Implicated in Multiple System Atrophy (MSA) and Acute Exposure to Endosulfan

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Pesticides and other environmental factors are known to modulate the pathogenesis of multiple system atrophy (MSA), a sporadic disease with alpha synuclein (α-Syn) implicated in its etiology. Aggregation of α-Syn within glial cytoplasmic inclusions (GCIs) in oligodendrocytes is a hallmark of MSA. However, the identity of environmental risk factors and how they interact with α-Syn and GCIs remain ill-defined. Recognizing the similarities between MSA pathophysiology and the neurotoxicology of various pesticides, we hypothesized that they may exhibit disease-toxicant interactions revealing cellular pathways underlying neurodegeneration. Here we utilize pesticides and OLN-93 rat oligodendroglial cell model of MSA to perform a gene-environment interaction screen. We report that oligodendroglial cells expressing mutant forms of α-Syn associated with familial cases of MSA, A53E-α-Syn and G51D-α-Syn, exhibit reduced sensitivity to endosulfan toxicity when compared to wild-type (WT-α-Syn) following 24 h exposure. This neuroprotective gene-environment interaction with endosulfan pesticide is highly specific, as it does not occur with dieldrin, mancozeb, chlorpyrifos, and MPP+. Analysis of the oxidative stress signaling pathway showed decreased production of reactive oxygen species and lipid peroxidation with endosulfan exposure in the mutant cells compared to WT-α-Syn. Indirect examination of cellular bioenergetics revealed that G51D-α-Syn cells show neuroprotection against endosulfan-induced reduction of mitochondrial membrane potential compared to WT-α-Syn. Additionally, the activity of caspase-3 and caspase-9 are significantly altered in A53E-α-Syn mutant cells with endosulfan exposure. Thus, this disease-toxicant interaction screen has revealed that expression of mutant α-Syn, A53E-α-Syn and G51D-α-Syn, results in reduced sensitivity to endosulfan neurotoxicity through distinct cellular pathways.

3038 Protection against Parathion-Induced Neurotoxicity by a Menadione/Vitamin C Combination Drug

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Accidental or intentional exposures to parathion, an organophosphorus (OP) pesticide, can cause severe poisoning in humans. Parathion toxicity is dependent on its metabolism by the cytochrome P450 system to paraoxon (diethyl 4-nitrophenyl phosphate), a highly poisonous nerve agent and potent inhibitor of acetylcholinesterase (AChE). Exposure to paraoxon can result in tremors, convulsions, dyspnea, respiratory arrest and unconsciousness. Previously, we reported that menadione, which functions by reducing cytochrome P450-mediated metabolic activation of parathion to paraoxon, significantly reduced lethality and overall toxicity of parathion in a rat model. Earlier studies have suggested that vitamin C may stabilize menadione; a mix of menadione/vitamin C is also undergoing clinical trials for cancer. In the present studies, we examined the effects of vitamin C on the ability of menadione to mitigate parathion toxicity. We found that vitamin C did not alter menadione-mediated cytochrome P450 inhibition using recombinant CYP1A2 and CYP3A4. It also did not interfere with the ability of menadione to suppress paraoxon formation and inhibition of AChE. Thus, a menadione/vitamin C mix significantly reduced parathion metabolism by liver microsomes in a concentration-dependent manner. This mix increased the IC_{50} values of AChE inhibition after parathion activation by rat liver microsomes from 0.8 to 2.7 μM and human liver microsomes from 1.9 to 81.6 μM, when compared to parathion. Administration of menadione/vitamin C to rats significantly increased parathion levels in serum, reduced parathion neurotoxicity and prolonged animal survival, similar to those found in menadione-treated rats. Taken together, these data indicate that vitamin C does not alter the protective effects of menadione against parathion intoxication and a menadione/vitamin C mix might be an effective therapeutic candidate to mitigate parathion-induced neurotoxicity. *Support: NIH grants AR055073, NS108956, and ES005022.*

3039 Evaluation of Serotonin-Modifying Toxicants Using a Standardized Tracking and Behavioral Model in *C. elegans*

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CPF is a commonly used organophosphate pesticide in the US despite evidence that it can cause life-long, persistent changes in the serotonergic system of mammals. Serotonin is responsible for modulating behavior and plays an important role in behavioral plasticity, memory, and learning. Here, we use the model system *C. elegans* as it shows a high degree of conservation of the serotonergic system with mammals including serotonin receptor, transporter, and synthesis and shows alterations in behavior in response to serotonin deficiency. We will evaluate this pesticide, CPF, using a self-designed and standardized behavioral model for serotonin-mediated behaviors, and monitor its effects on behaviors and alterations to the serotonergic system. We will also evaluate how this exposure can influence gene expression and epigenetic mechanisms transgenerationally. We hypothesize that CPF will alter se-

rotonin-controlled behaviors and the serotonergic system, and that this will be seen transgenerationally and is influenced by changes in gene expression and epigenetic mechanisms. We have characterized the serotonin-controlled behaviors of enhanced slowing responses (ESR) via food deprivation, and basal slowing responses (BSR) under normal conditions for both wild type worms and mutant strains deficient in serotonin, dopamine, or both. Wild type worms display a significantly slower locomotion rate in their ($P < 0.0001$) when compared with their BSR by measuring either center point speed (fold change= 1.9) or absolute peristaltic speed (fold change 1.5). Other new insights into the behavior of mutants such as *mod-5* (deficient in serotonin) and *cat-2* (deficient in dopamine) reveal that *mod-5* wavelength shows no difference when compared to wild type for the BSR, while *cat-2* shows significantly (ESR = 1.27 fold change, BSR= 2.6 fold change) higher wavelength in both ESR and BSR($P < 0.0001$). This in-depth individual behavioral analysis and subsequent dose response study using our behavioral model we have developed is the first of its kind and will address a significant gap of knowledge into the transgenerational effects of serotonin modifying agents on behavior.

3040 Ultrastructural and Biochemical Alterations in Maneb-Treated Rat Hippocampal Astrocytes

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Maneb is a broad-spectrum manganese containing dithiocarbamate fungicide commonly used to control plant diseases on food and feed crops. There is increasing evidence that agricultural chemicals and other environmental contaminants, including metals such as manganese, are implicated in neurodegenerative diseases. In addition, there are several lines of evidence suggesting that Maneb exposure may play a role in the development of Parkinson's disease. Astrocytes play a critical role in normal brain physiology. This study evaluated Maneb-induced biochemical and ultrastructural changes in rat hippocampal astrocytes. Rat hippocampal astrocytes were exposed to 13.5 μM (LC_{50}) of Maneb for 24 hours. Biochemically, Maneb significantly increased lipid peroxidation, glutathione oxidation, SOD activity, and HO-1 and SP-70 levels as well as altered the regulation of stress-responsive and antioxidant gene expression in rat hippocampal astrocytes. Furthermore, Maneb treated cells had significantly elevated intracellular manganese and copper levels. These biochemical changes triggered apoptotic cell death via the intrinsic pathway as measured by cytochrome c release and activation of caspase-9 and -3/-7. Apoptosis was further confirmed by cellular externalization of phosphatidylserine and TUNEL assay. Evaluation of Maneb-induced ultrastructural changes revealed distorted and swollen mitochondria characterized by dilated and disrupted cristae and matrix dilution when compared to controls. Myelin whorl formation and non-membrane bound lipid droplets were also observed. In addition, the rough endoplasmic reticulum appeared deranged and swollen. Nuclei often appeared abnormal with a distorted shape and condensed chromatin. This study provides direct biochemical and ultrastructural evidence of Maneb-mediated mitochondrial toxicity in mammalian astrocytes.

3041 Mechanisms of Chlorpyrifos-Driven Programmed Cell Death in Neurons

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Age-related neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, are characterized by progressive neuronal loss observable in multiple brain regions. A small percentage of these cases can be attributed to genetic mutations however, the majority arise from unknown causes. Exposure to environmental toxicants, such as pesticides and heavy metals, is strongly associated with an increased risk of developing age-related neurodegenerative diseases. These epidemiologic data emphasis the need to characterize the molecular mechanisms underlying increased risk associated with widely used toxicants. Such foundational studies are anticipated to improve our understanding of the initiation and progression of neurologic disorders and inform the development of new treatments. Chlorpyrifos (CPF) is a widely used, commercially available, blood-brain-barrier permeant organophosphate pesticide. Exposure to CPF is associated with increased risk of developing multiple age-related neurodegenerative diseases, but the mechanisms of toxicity underlying CPF exposure in neurons are not completely understood. We conducted unbiased transcriptomic and ontologic analyses of CPF-exposed primary neuron cultures seeking pathways characterizing CPF-induced neurotoxicity. These studies identified the pro-apoptotic mediator *Bbc3/Puma* (Bcl-2 binding component-3/*p53* upregulated modulator of *a*poptosis) and other transcripts related to the greater *p53* pathway, as significantly elevated as the result of CPF exposure compared to vehicle-treated controls. We next cultivated primary cortical neurons isolated from WT and *Bbc3*$^{-/-}$ mice and discovered the IC_{50} of CPF to be significantly higher in neurons lacking *Bbc3* compared with WT controls. Real-time PCR analysis confirmed up-regulation of *Bbc3*, *p53*, and the *p53*-apoptotic pathway related gene *Pmaip1* (encoding the NOXA protein) resulting from CPF exposure in WT neuronal cultures, that was not observed in CPF-treated *Bbc3*$^{-/-}$ cultures. Further characterization of CPF-exposed WT and *Bbc3*$^{-/-}$ cultures identified cleavage of caspase 3 and PARP in WT cultures exposed to CPF, but not in identically treated *Bbc3*$^{-/-}$ cultures. Data suggest that *Bbc3* is a key node in a programmed cell death pathway initiated in neurons resulting from CPF exposure. Studies provide experimental evidence to support epidemiologic findings related to CPF and a platform for further analysis of cellular mechanisms related to disease-associated pesticides on the brain.

3042 A Cell Viability Study on the Effects of Glyphosate, Mancozeb, and Their Combination on Mouse Neuroblastoma Cells

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The extensive application of pesticides results in increased human exposure to complex mixtures of these compounds. The toxicological effects of pesticides have long been studied. Most of the studies however are concerned with single pesticide exposures and there is a lack of information regarding the toxicity of pesticide mixtures. In this study we are examining the combination of two of the most widely used pesticides, Glyphosate and Mancozeb. Glyphosate is a systemic non-selective herbicide. Since its introduction in the 1970s, Glyphosate has been considered safe to mammals. Recent evidence however links Glyphosate to multiple adverse human health effects. Mancozeb is a broad-spectrum fungicide. Mancozeb has been associated with neurotoxicity, developmental toxicity, immunotoxicity and carcinogenicity. In this study we investigated the toxicity of Glyphosate and Mancozeb alone and in combination on the viability of mouse neuroblastoma (Neuro- 2A) cells. Cell viability was assessed using Trypan Blue Exclusion Assay. Neuro-2A cells were treated for 24 hours with Glyphosate alone (100μM, 500μM, 1000μM, 5000μM and 10,000μM) or Mancozeb alone (2μM, 4μM, 6μM, 8μM and 10μM). Glyphosate and Mancozeb treatments resulted in a significant reduction in cell viability at all concentrations when compared to control except Glyphosate 100μM and Mancozeb 2μM. Cells were also treated with combination of Glyphosate (100μM) and Mancozeb (6μM). Three forms of combination were used including Mancozeb (30 minutes) followed by Glyphosate (24 hours), Glyphosate (30 minutes) followed by Mancozeb (24 hours) or Glyphosate plus Mancozeb (24 hours). Glyphosate and Mancozeb combination treatments resulted in a statistically significant reduction in cell viability when compared to Glyphosate alone and Mancozeb alone treatments except for Glyphosate plus Mancozeb (24 hours) combination treatment, which was only statistically significant from Glyphosate alone treatments. From these results we concluded that Glyphosate and Mancozeb combinations, Mancozeb (30 minutes) followed by Glyphosate (24 hours) as well as Glyphosate (30 minutes) followed by Mancozeb (24 hours) are more toxic than Glyphosate alone and Mancozeb alone on the viability of Neuro-2A cells.

3043 Inhibition of Neurite Outgrowth upon Exposure to Chlorpyrifos

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Chlorpyrifos is an organophosphate insecticide that has been shown to have toxic effects on the human nervous system. Research suggests that chronic, low dose exposure to chlorpyrifos contributes to Parkinson's disease and interferes with neurodevelopment. It was hypothesized that if dopaminergic neurons in culture were exposed to 2 μM, 10 μM, and 20 μM chlorpyrifos for 24 hours, then neurite outgrowth would decrease with increasing concentration. Cells were exposed to three concentrations of chlorpyrifos (2 μM, 10 μM, or 20 μM) and a control (0.02% ethanol). After 24 hours, the cells were fixed and microscopic images of neurites were captured. The results indicate that there was a concentration-dependent decrease in neurite outgrowth between the control and 2uM chlorpyrifos in comparison to both 10 μM and 20 μM chlorpyrifos. Finally, there was a significant decrease in neurite outgrowth between 10 μM and 20 μM chlorpyrifos. To investigate the role of specific proteins contributing to this effect, Western blot analysis on cell lysates was conducted. Analysis of the blots indicated no significant difference in α-tubulin concentration or PKC concentration. The data indicate that increased concentrations of chlorpyrifos result in significantly decreased neurite outgrowth but that neither α-tubulin nor PKC are significantly suppressed during this event. Decreased neurite outgrowth likely contributes to decreased neurotransmission of dopaminergic neurons, thereby contributing to the Parkinson's disease and delayed neurodevelopment.

3044 Mylein Degeneration Is Accompanied by Differential mRNA Expression of Basic Myelin Protein in the Central Nervous System of Hens Treated with Diisopropyl Phosphorofluoridate (DFP)

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Diisopropyl phosphorofluoridate (DFP) produces organophosphorus-ester induced delayed neurotoxicity (OPIDN) in the hen, human and other sensitive species. We studied the effect of single dose of DFP (1.7 mg/kg/s.c.) on the level of myelin degeneration by advanced histological quantitative techniques and mRNA expression of Myelin Basic Protein (MBP) which is one of the components of myelin sheath by northern blotting. The hens were sacrificed at different time points i.e. 1, 2, 5, 10, and 20 days. Total RNA was extracted from the following brain regions: cerebrum, cerebellum, brainstem, and spinal cord. Northern blots prepared using standard protocols were hybridized with MBP as well as with beta-actin and 28S RNA cDNA (controls) probes. The results indicate a differential/spatial/temporal regulation of MBP levels in the highly susceptible tissues like brainstem and spinal cord while the resistant tissue cerebrum tissue showed generally increased or control levels in various time points. Histologically, the greatest amount of myelin disintegration was noted in the ventral tract caudally and in the dorso-ventral tract rostrally. Major degeneration of the myelin sheath was noted at 10 days post treatment and beyond. Two phases of myelin disintegration namely a) contraction myelin sheath and b) sheath disintegration were noted during earlier and later time points respectively. The distribution of the damage in the CNS followed the lumbosacral region of spinal cord leading to the anterior (ventral tract). The spino-cerebellar tract as well as posterior columns in the upper thoracic and cervical regions also showed myelin degeneration. Hence our results indicate differential expression of MPB accompanied by degeneration of myelin sheath is either one of the reasons for the development of OPIDN or the result of progressive changes taking place during OPIDN.

3045 Effects of Cyfluthrin on Gene Expression Profiling of Oxidative Stress Pathways in SH-SY5Y Cells

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Exposure of humans to chemicals that negatively affects CNS is of concern. The worldwide application of pyrethroid insecticides necessitates an appraisal of their potential hazards to man and animals. Oxidative stress has been postulated to be one of the key contributors to dopaminergic neurodegeneration. The human dopaminergic neuroblastoma cell line, SH-SY5Y, is a commonly used cell line in studies related to neurotoxicity, oxidative stress, and neurodegenerative diseases. In this study, we show that the treatment of SH-SY5Y cells with the pyrethroid insecticide cyfluthrin in a dose dependent manner (1-25 μM) after 24 h incubation period, led to a robust cellular ROS formation and an increase of MDA and NO levels. Gene expressions linked to apoptosis, proinflammation and oxidative stress also were evaluated. Quantitative Real-Time PCR assays for Bax, Bcl-2, Casp-3, BNIP3, AKT1, p53, APAF1, NFκB1, TNFα, and Nrf2 mRNA were performed to analyze mRNA gene expressions. Moreover, this study analyzed, by Real-Time PCR array, the expression of 84 key genes related to oxidative stress (antioxidants, ROS metabolism and pathway activity signature genes) after cyfluthrin exposure. It was observed in the RNA samples from cyfluthrin treated SH-SY5Y cells a greater or lower fold change than 3 in comparison with the control in the expression of the 13 genes included in PCR array. Our results showed that the major over-expressed genes by cyfluthrin were CYBB, DUOX1, DUOX2, AOX1, BNIP3, HSPA1A and NOS2. The major over-expression was detected for CYBB gene (7.35-fold); melatonin (1 μM) provided a significant decrease on mRNA expression level of CYBB (7.35-fold to 2.53-fold). According to our experimental data, the possibility to use measurement of CYBB expression as an oxidative biomarker of the pyrethroid intoxication is a key to be clarified. *Work supported by Project Ref. RTA2015-00010-C03-03 from Ministerio de Economía, Industria y Competitividad, Spain.*

3046 Interethnic Human Differences in the Biotransformation of Chlorpyrifos and Resulting Acetylcholinesterase Inhibition

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Chlorpyrifos (CPF) is a well-known insecticide used all over the world. The acute toxicity upon exposure to CPF is due to inhibition of acetylcholinesterase (AChE). The aim of the present study was to assess the interethnic differences in the biotransformation of CPF and resulting red blood cell (RBC) AChE inhibition in both the Chinese and the Caucasian population by using ethnic-specific physiologically based kinetic (PBK) models for CPF and reverse dosimetry. In this way, an *in vitro* concentration response curve for RBC AChE inhibition was converted to an *in vivo* dose response curve for both populations. The extrapolated RBC AChE inhibition dose response curve revealed that the Caucasians were predicted to be 4- to 7-fold more sensitive to CPF-induced *in vivo* RBC AChE inhibition than the Chinese. This interethnic difference was mainly due to the fact that the Caucasians appear to be more efficient in the bioactivation of CPF to chlorpyrifos-oxon (CPO) but less efficient in detoxification of CPO to 3,5,6-trichloro-2-pyridinol (TCPy). Altogether, the developed ethnic-specific PBK models for CPF can predict the human interethnic differences in sensitivity towards CPF exposure, with the Caucasians being more sensitive than the Chinese showing similar endogenous levels of CPO and AChE inhibition at lower dose levels.

3047 Spatiotemporal Phosphoprotein Signaling in a Mouse Model Using Corticosterone and Relevant Organophosphates

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Each year, 3 million people are exposed to organophosphates, contributing to approximately 300,000 deaths; many do not report appreciable acute effects immediately following exposure, but report symptoms of adverse neurological effects years later, as is the case for the 250,000 veterans from the 1991 Persian Gulf War who suffer from Gulf War Illness (GWI). Previous GWI research has focused on models that dose with acetylcholinesterase (AChE) inhibitors (chemical warfare agents and pesticides) and exogenous corticosterone (CORT), to simulate high stress, in efforts to mimic chronic neuroinflammation associated with GWI symptomology. An investigation into early phosphoprotein responses in the cortex and striatum were performed to better understand the similarities of the early cellular changes involved in these etiologies. Using a mouse model, adult male C57BL/6J mice were exposed to CORT in the drinking water for 7 days followed by a single injection of diisopropyl fluorophosphate (DFP; 4.0 mg/kg, i.p.) or chlorpyrifos oxon (CPO; 8.0 mg/kg, i.p.) on day 8 and euthanized 30 min, 2 h, and 24 h post-injection via focused microwave irradiation. To evaluate brain-region-specific effects, 20+ post-translationally modified protein targets were measured using a multiplex ELISA (e.g., ERK1/2, IkB-a, JNK, MEK1) for both dosing regimens. Several phosphoprotein responses (ZAP70 and JNK) were found to be significantly increased ($p<0.05$) for CORT+CPO exposure, but not for CORT+DFP in both brain regions. Conversely, CREB and ERK were significantly phosphorylated for DFP exposures relative to CPO. This suggests the neuroinflammatory response in this mouse model may be driven by off-target mechanisms of AChE exposure. Further investigation of other relevant exposures and their phosphoprotein signaling effects must be performed to better understand potential biomolecular drivers of GWI-like symptomology.

3048 RNA-seq Evaluation of DDT Exposure in the Hippocampus of Humanized APOE Mice

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The Apolipoprotein 4 (APOE) gene variant is the strongest genetic risk factor for late-onset Alzheimer's disease (LOAD). However, it is not entirely predictive of LOAD, and emerging evidence points to environmental factors in the etiology of disease. We have previously reported that individuals with increased levels of the primary metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT) and harboring an APOE4 allele performed worse on a task of cognitive function. In the present study, we utilized male and female targeted replacement humanized APOE3 (E3) and APOE4 (E4) mice to identify the effects of DDT exposure on gene expression changes in the

hippocampus. Three-month-old E3 and E4 mice were exposed to 3 mg/kg DDT by oral gavage every 3 days for 5 months. Animals were euthanized at 8 months and RNA-sequencing was performed on hippocampal samples using the NovaSeq 6000. Data were analyzed using DESeq2 in R. Contrasts were made within each genotype across sex, between the genotypes, and across exposure groups. Each contrast underwent a Wald test, and a likelihood-ratio test for significance and only significant transcripts were used for g:profiler analysis. Between males and females, within each genotype, there were 13 (E3) and 36 (E4) differentially regulated biological process pathways. Between each genotype, within each sex, there was 1 (males) and 49 (females) differentially regulated biological process pathways. There were no significantly altered biological processes in any of the DDT exposed animals compared to their respective control within each sex and genotype. However, when analysis was restricted to DDT-treated E4 and E3 males, there were a total of 51 downregulated biological process pathways in the E4 males compared to the E3 males. These pathways included cell-cell signaling ($P_{adj}=1.1\times10^{-3}$), chemical synaptic transmission ($P_{adj}=7.3\times10^{-3}$), regulation of cation channel activity ($P_{adj}=1.5\times10^{-2}$) and regulation of the action potential ($P_{adj}=1.8\times10^{-2}$). These data suggest a gene x environment interaction with exposure to DDT and the APOE4 genotype, that occurs exclusively in males and affects pathways consistent to the known effects of DDT on the neuron. *Supported in part by NIH R01ES026057.*

PS 3049 Epigenetic Histone Acetylation and BDNF Dysregulation in a Rat Model for Gulf War Illness

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Around 35% of the returning soldiers from the First Gulf War exhibit chronic multi-symptom illness also known as the Gulf War Illness (GWI), chief among which are the neurological morbidities of depression, mood disorders, and cognitive impairment. While the causes for GWI are known, the persistence of GWI is still not fully understood. Epigenetic mechanisms respond to external stimuli such as the environment and experiences, and the effects of such exposures can become embedded in the genome to produce long-lasting changes in cellular regulation. Epigenetic processes include chemical modifications including DNA methylation and histone acetylation and deacetylation. DNA methylation changes have been reported in GWI, however, the role of histone modifications in GWI is ill understood despite its importance as a major epigenetic mechanism for neurological disorders. Male Sprague-Dawley rats (3-m, n= 30) were exposed to DFP (0.5 mg/kg s.c., 5-d) or ice-cold PBS and, 6-m later assessed for mood and memory function using a battery of rat behavioral assays. Rats were then sacrificed and brains harvested for protein studies and epigenetic analyses. Histone acetylation and deacetylation is catalyzed by two enzymes: histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity. Antibody-specific western blotting studies revealed a significant upregulation in HDAC1 protein in GWI rats compared to age-matched control rats indicative of possible increase in HDAC activity in GWI. The brain derived neurotrophic factor (*Bdnf*) gene has been implicated in many neurological disorders that share symptomatology with GWI neurological symptoms. We conducted a locus-specific study of this epigenetic modification using chromatin immunoprecipitation (ChIP) and observed decreases in H3K9ac at the *Bdnf* promoter adjacent to exon 4. Such a decreased histone acetylation would indicate lower levels of *Bdnf* gene transcription. Indeed, a significant decrease in Bdnf protein was subsequently noted in GWI rat hippocampus compared to age-matched control rats (n= 8 rats/condition, t-test, $p<0.05$). Increased HDAC activity and aberrant BDNF expression could underlie pathological synaptic plasticity in GWI rat model that expresses itself as GWI neurological symptoms. Our studies further indicate that HDAC inhibitors could prove to be effective therapies for GWI behavioral symptoms. These studies are currently underway in our laboratory.

PS 3050 Development and Validation of a Simple High-Resolution Liquid Chromatography-Orbitrap Mass Spectrometry Method for the Determination of Six Strobilurin Fungicides in Mouse Brain Tissue

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Strobilurin fungicides are a class of broad-spectrum fungicides widely used to control diseases, boost yields, and improve quality/production of crops in modern agriculture. Although they are bio-degraded in about 21 days, the inappropriate and widespread use, and high residue rates of these chemicals, may cause high exposure and potential toxicity to humans. These fungicides are of neuro, reproductive, developmental, and immune toxicity concern. To better explore the potential toxicity of these substances, a fast and simple analytical approach is essential. In this study, a high-resolution liquid chromatography orbitrap mass spectrometry method was developed and validated to determinate six strobilurin fungicides, azoxystrobin, kresoxim-methyl, picoxystrobin, trifloxystrobin, dimoxystrobin, and fluoxastrobin in mouse brain tissue. About 20 mg of brain tissue was accurately weighed into a lysing matrix tube (MP Biomedicals, lysing matrix D) and then homogenized after adding water and acetonitrile in a volume ratio of 20/80. Samples were then centrifuged and the supernatant was removed for analysis. The samples were separated on a Thermo Hypersil Gold aQ (100 × 2.1mm, 1.9µm) column using an isocratic method with mobile phase consisting of water and acetonitrile (63/27), containing 0.1% formic acid. The flow rate was 0.3 mL/min with 4 min of total run time. Target compounds were detected on a Q-Exactive Orbitrap mass spectrometry in targeted-SIM/dd-MS^2 mode. The method was validated in terms of limit of detection (LOD), limit of quantitation (LOQ), linearity, precision, accuracy and matrix effects. The LODs and LOQs were about 0.02 ng/mL and 0.1 ng/mL based on 3 and 10 times of signal-to-noise, respectively. The linearity is excellent with a $R^2 > 0.98$ over the concentration range of 1.0 - 100 ng/mL. The recoveries and matrix effects were 91.4 -115.5% and 82.6-108.0%, respectively. The method is precise with an RSD < 15%. The method was applied to analysis of six strobilurin fungicides in mouse brain tissue after dosing and showed good performance. This approach is very simple, fast and sensitive, and will be very helpful for further toxicological studies of strobilurin fungicides.

PS 3051 Mitochondrial Dysfunction Induces Epigenetic Dysregulation by H3K27 Hyperacetylation to Perturb Active Enhancers in Parkinson's Disease Models

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Genetic mutations explain only 10-15% of cases of Parkinson's disease (PD), while an overriding environmental component has been implicated in the etiopathogenesis of PD. But regardless of where the underlying triggers for the onset of familial and sporadic PD fall on the gene-environment axis, mitochondrial dysfunction emerges as a common mediator of dopaminergic neuronal degeneration. Herein, we employ a multidisciplinary approach to convincingly demonstrate that neurotoxicant exposure- and genetic mutation-driven mitochondrial dysfunction share a common mechanism of epigenetic dysregulation. Under both scenarios, lysine 27 acetylation of likely variant H3.2 (H3.2K27ac) increased in dopaminergic neuronal models of PD, thereby opening that region to active enhancer activity via H3K27 hyperacetylation. These vulnerable epigenomic loci represent potential transcription factor motifs for PD pathogenesis. We further confirmed the mitochondrial dysfunction induced H3K27ac during neurodegeneration in *ex vivo* models of PD. Our results reveal an exciting axis of 'exposure/mutation-mitochondrial dysfunction-metabolism-H3K27ac-transcriptome' for PD pathogenesis. Collectively, the novel mechanistic insights presented here interlinks mitochondrial dysfunction to epigenetic transcriptional regulation in dopaminergic degeneration as well as offer potential new epigenetic intervention strategies for PD. *Support: R01ES027245, R01ES026892, R01NS100090 and R01NS088206, Eugene and Linda Lloyd Endowment and Armbrust Endowment.*

PS 3052 Bisphenol A Neurotoxicity in Human Neuroblastoma Cells: Role of Alpha-Synuclein Aggregation and Mitochondrial Dysfunction

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Bisphenol A (BPA) is considered a major public health concern. Exposure to even a low dose of BPA has been linked to diverse negative pathological, cellular, and molecular effects. Synucleinopathies are a group of human neurodegenerative diseases characterized by the accumulation of α-synuclein in various regions of the central and peripheral nervous system. α-Synuclein is involved in the pathogenesis of misfolding-related neurodegenerative diseases, in particular Parkinson's disease. A misfolding event leads to the formation of oligomers, which are believed to result in cell toxicity and eventually lead to the death of neuronal cells. Neurodegeneration has increasingly been associated with mitochondrial dysfunction and inhibition of the electron transport chain. Neurons require high levels of energy in order to operate. The production of ATP in neuronal cells may be reduced due to mitochondrial dysfunction. Direct association of α-synuclein with mitochondria has been repeatedly and constantly observed in model cells and in different brain re-

gions of the transgenic mouse model. This study was aimed at examining the cellular and molecular mechanisms that might underlie BPA-induced neurodegeneration. Human neuroblastoma SH-SY5Y cells were exposed to various concentrations of BPA in the presence and absence of α-synuclein. Cell viability, mitochondrial dysfunction, and α-synuclein intracellular aggregation were evaluated. Exposure to BPA for 3h resulted in a significant decrease of mitochondrial membrane potential similar to CCCP, the mitochondrial uncoupler positive control. The cellular level of ATP was significantly reduced after exposure to BPA for 24h. Incubation of α-synuclein with increasing BPA concentrations *in vitro* resulted in a progressive acceleration of α-synuclein-BPA complex formation in a concentration-dependent manner. Treatment of SH-SY5Y cells for 48h with α-synuclein showed significant neurotoxic effect with cell death. Furthermore, BPA-treated cells showed increased level of α-synuclein aggregation indicating that the ability of BPA to promote aggregation may contribute to neurodegeneration. These results suggest that impairment of mitochondrial function and enhancement of α-synuclein aggregation represent potential mechanisms underlying BPA neurotoxicity. *Supported by Title III.*

3053 Power Spectrum Analysis of EEG in a Translational Nonhuman Primate Model after Chronic Exposure to Low Levels of the Common Marine Neurotoxin Domoic Acid

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Domoic acid (DA), the focus of this research, is a marine algal neurotoxin and epileptogen produced by species in the genus *Pseudo-nitzschia* and found in finfish and shellfish across the globe. The current regulatory limit for DA is set to protect humans from acute toxic effects, but there is a growing body of evidence suggesting that regular consumption of DA contaminated seafood at or below the regulatory limit may lead to subtle health effects in young children and older adults. In previous research from our laboratory, a translational nonhuman primate model was used to assess the reproductive and neurodevelopmental effects of chronic DA exposure near the regulatory limit. Healthy adult female *Macaca fascicularis*, orally administered DA at levels near the current regulatory limit (0.075 and 0.15 mg/kg/day) for at least 9 months, showed signs of unanticipated neurotoxicity; intention tremors while performing a reaching task. This neurobehavioral effect was significantly related to the DA dose, but the biological underpinnings of the behavior were largely unknown. The present research is the first to investigate how *in vivo* neurological function is related to chronic DA exposure and DA-intention tremors, using sedated, quantitative electroencephalography (qEEG). In the EEG recordings, we captured the never before documented electrooculographic (EOG) artifacts in this macaque species. Analysis of dose-response relationships suggests that neuroelectrical function of the 0.15 mg/kg group is significantly decreased in the delta range (1-4 Hz, $p < 0.05$), as well as significantly increased in the theta range (5-8 Hz, $p<0.05$). Tremors, however, were not associated with any measures of the power spectrum, suggesting that the observed neurotoxicity may not be early signs of epileptic activity that results from acute DA exposures. This research is the first to utilize *in vivo* EEGs in a translational, noninvasive toxicological primate study, showcasing how EEG can be successfully implemented on the small nonhuman primate model. Results from the study reveal new insight into our understanding of DA toxicities after exposure to levels found in the real-world and will be an important component of DA research needed to best protect public health across the globe.

3054 Chemical Screen of Human iPSC-Derived Neurons for Mitochondrial Dysfunction

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Neurons have high metabolic rates that increase their vulnerablity to toxic mitochondrial insult, resulting in cell dysfunction and death due to reactive oxygen species (ROS) generation and insufficient levels of energy. Here, we report a high throughput, first line evaluation based on hiPSC-derived neurons to identify chemicals that cause mitochondrial dysfunction. To test this approach, a library of 73 suspected or known neurotoxic chemicals was assembled, representing diverse chemical classes. Chemicals were selected from ToxCast/Tox21 to allow comparisons with public data bases, and to support quantitative structure activity relationship (QSAR) modeling. We utilized iCell neurons (Fuji Film Cellular Dynamics, Inc.), an hiPSC-derived population of primarily GABAergic neurons. High-content image-based screening was performed in dose response, on 384 well-plates using a live cell, acute exposure, multiplexed assay for mitochondrial membrane potential (MMP) and generation of ROS. In parallel, we also screened for effects on neurite growth in a fixed cell assay. For mitochondrial and neurite assays, nuclear parameters, count, intensity, area, and shape, were monitored as measures of cytotoxicity. Of the 73 chemicals tested, we identified 34 hits in the mitochondrial assay, 3 of which were also identified as hits in the neurite assay. Hits depolarized MMP and/or increased ROS at concentrations lower than those that decreased cell viability based on nuclear measures. Our hit set contains both chemicals that were previously reported to be mitochondrial disrupters, as well as those that are novel. Companion QSAR mitochondrial toxicity models built on a ~7000 compound Tox21 mitochondrial toxicity library was applied to these results and had a high predictability for compounds screened in this human neuron assay system. Future development of this scalable source of human neurons, in terms of neuronal sub-type, and complexity of phenotypic read-outs, will provide a powerful approach to determine neurotoxicity of large collections of chemicals, especially when combined with *in silico* QSAR analyses.

3055 Altered BCRP Transport Activity at the Blood-Brain Barrier following 2,4,6-Tribromophenol Exposure

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The brominated chemical, 2,4,6-Tribromophenol (TBP, CAS No. 118-79-6), is used in the production of flame-retardant epoxy resins and wood preservatives. TBP is a byproduct released into the environment by food processing and water treatment facilities. TBP is found in shellfish and predatory fish and in humans TBP has been detected in blood and breast milk. Mechanistically, TBP interferes with estrogen and thyroid hormone signaling pathways, which are known regulators of ATP binding cassette (ABC) transporters of the blood-brain barrier (BBB). The BBB resides in the brain microvasculature is a highly regulated two-component system containing tight-junction proteins and ABC efflux transporters. Using a steady-state confocal microscopy-based assay, this study examined the effects of TBP (0.1 nM-100 nM) on the transport activity and expression of Breast Cancer Resistance Protein (BCRP), and Multidrug Resistance Associated Protein 2 (MRP2) at the BBB. BCRP and MRP2 transport activities were measured in rat brain capillaries after *ex vivo* and *in vivo* TBP exposures. Freshly isolated rat brain capillaries treated *ex vivo* with TBP (0.1-100 nM) for 4 h produced no significant changes in MRP2 transport activity in either sex. However, exposing capillaries *ex vivo* to similar TBP concentrations significantly decreased BCRP transport activity in both males and females. Time course studies revealed that the TBP mediated decreases in BCRP transport activity in both males and females occurred two hours after TBP (100nM) exposure. *In vivo* TBP dosing by oral gavage at 30 pmol/kg, 300pmol/kg, and 3nmol/kg significantly decreased BCRP transport activity in both sexes relative to vehicle controls. Immunochemistry and western blots analysis of brain capillaries revealed no changes in BCRP protein levels in TBP treated compared to untreated controls. This work shows that relatively low concentrations of TBP (0.1-100 nM) alters ABC transporter activity at the BBB which can disrupt brain homeostasis, hinder drug delivery, and increase the likelihood of CNS exposure to harmful xenobiotic toxicants. *This research was supported by the Intramural Research Program of NIH/NCI [Project ZIA BC 011476] and does not necessarily represent US EPA policy.*

3056 Repeated Exposure of 3,4-Dichloro-1-Butene Leads to Decreased Grip Strength and Alters Peripheral Nerve Function and Somatosensory Evoked Potentials in Adult Male Long-Evans Rats

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3,4-Dichloro-1-butene (DCB) is used in manufacturing rubber and cosmetics. An analysis using Hard-Soft Acid-Base (HSAB) concepts predicted that DCB will react with cellular proteins and potentially cause neurotoxicity after repeated exposure. Adult male Long-Evans rats were treated with DCB via oral gavage (corn oil vehicle) using concentrations of 0, 100, 175, or 225 mg/kg/day for 8 weeks. Behavioral observations (open field activity, foot splay, grip strength) were conducted weekly. Peripheral nerve function was evaluated *in vivo* with nerve excitability testing, consisting of compound muscle action potential (CMAP) or nerve action potential recordings by stimulating the tail (motor and mixed) nerves and sciatic (motor) nerve. Compound nerve action potentials (CNAP) and nerve conduction velocity (NCV; tail nerves) were assessed *in vivo*, along with somatosensory function by recording evoked potentials (SEPs) over the cortex and cerebellum. Animals treated with DCB had a dose-related suppression in weight-gain compared to controls throughout the study. Behavioral tests indicated a reduction in forelimb and hindlimb

grip strength in the high-dose group. At all doses, nerve excitability data showed an effect on tail mixed nerve action potentials, suggesting alterations to nerve fibers that were consistent with neuronal depolarization (altered Na^+ conduction) and possible K^+ channel activity. In the high-dose group, CNAP recordings showed an increase in amplitude and NCV, possibly due to a loss of small nerve fibers. A reduction in latency was seen in the SEP cerebellum recordings, suggesting faster conduction and/or less inhibitory input. These changes further support the possibility that small afferent nerve fibers were altered. DCB treatment was associated with the development of an additional peak (N1-P1) at the front of the somatosensory cortical response, changes which could be related to effects at the cortical level or possibly a splitting of the afferent volley. Overall, this data indicates that treatment with DCB over this dose range and duration influences peripheral nerve and somatosensory function. Future studies should include histopathology to assess nerve fiber degeneration. *This is an abstract of a proposed presentation and does not necessarily reflect US EPA policy*.

PS 3057 Exploring the Effects of Developmental Exposure to Flame Retardant Mixture and Components on Adult Wistar Rat Behavior

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FireMaster 550 (FM550) is one of the most commonly used fire retardants (FR) on foam-based furniture and baby products. This commercial mixture includes 2 brominated compounds, 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) mixed with bis (2-ethylhexyl) tetrabromophthalate (BEH-TEBP), and two organophosphates, triphenyl phosphate (TPhP) and assorted isopropyl triphenylphosphate (ITP) isomers, in the ratio: 36% EH-TBB, 14% BEH-TEBP, 18% TPhP and 32% ITPs by weight. As an additive FR, components of FM550 are readily leaching from consumer goods with detectable levels found globally in indoor dust, indoor/outdoor air, aquatic biota and food leading to widespread human exposure. Due to the structural similarities with known neurotoxicants and endocrine-disrupting chemicals (EDCs), including polybrominated diphenyl ethers (PBDE) and organophosphate pesticides, this is of great concern. Previously our lab demonstrated sex specific behavioral outcomes following developmental exposure to FM550 with males displaying increased anxiety and females being hyperactive. We are now exploring the relative contributions of the FM550 components on vulnerable brain and placental endpoints. The present, ongoing, studies focus on adult behaviors. Female Wistar rats were exposed to either vehicle, 1000µg brominated mixture (EH-TBB and BEH-TEBP), 1000µg ITP mixture or 2000µg FM550 mixture once daily from gestation day 0 to postnatal day 21. To monitor growth, all female and male offspring weights are collected regularly. Beginning on PND 65, at least one male and one female per litter are being evaluated using an array of behavior tests to assess repetitive impulsive behaviors, activity levels, social behaviors, anxiety-like behavior and short and long-term memory. Preliminary analysis suggests increased running wheel activity in males and females exposed to FM550 or the ITP mixture. These outcomes are helping to elucidate which FM 550 components contribute to sex-specific effects of exposure.

PS 3058 Addressing the Impact of Polychlorinated Biphenyl Environmental Mixtures on Ryanodine Receptor Activity

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Polychlorinated biphenyls (PCBs) are halogenated aromatic hydrocarbons with 209 congeners. They were commonly used in commercial and industrial products until their ban in 1979 due to the rising concerns over their adverse effect on humans and the environment. Despite the ban, PCB congeners are still found in the air, in environmental water, on sediment, and in organismal and human samples. Some PCB congeners can alter the activity of ryanodine receptors (RyR), a Ca^{2+} channel important for neuronal and muscle cell Ca^{2+} homeostasis. Most studies focus on the effect of individual PCBs on RyR activity, but PCBs are found in mixtures in the environment. The aim of this study is to address the additivity of environmental mixtures of PCBs on RyR activity using radio-ligand binding assays. Currently, the binding assay was validated in a crude protein preparation and has confirmed that the highly active PCB 95 causes a 700% increase in activity at the ryanodine receptor. The development of a sucrose gradient is underway to further purify the crude protein in order to isolate the target ryanodine receptor. Based on previous research, the developed hypothesize predicts that PCBs will act additively towards RyRs, providing more insight into the influence of environmental mixtures on RyR based cellular pathways and organismal physiology. *Acknowledgements: This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers; UL1GM118979; TL4GM118980; RL5GM118978.*

PS 3059 Whole Genome Transcriptome Analysis in a Genetic Model of Gulf War Illness

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Gulf War illness (GWI) affected up to 30% of the nearly one million personnel sent to the Persian Gulf in 1991. The probable cause was exposure to organophosphorus compounds coupled with high circulating glucocorticoids as would be expected in a combat theater. Previously, we developed a mouse model consisting of 7 days of exposure to corticosterone in the drinking water followed by injection with diisopropylflurophosphate (DFP, surrogate for sarin) and assessment of pro-inflammatory cytokines in frontal cortex and hippocampus 6h after DFP treatment. In order to assess genetic-based individual differences in susceptibility to developing GWI, we applied the model to male and female C57BL/6J and DBA/2J mice. Consequently we observed wide-genetic differences in pro-inflammatory gene expression by qPCR in the prefrontal cortex. We then subjected the prefrontal cortex to genome-wide transcriptome response by RNA-seq, comparing the combined corticosterone-DFP treatment. Gene ontology analysis showed altered immune function and apoptosis as the top systems affected. We also verified a previous nomination of Spondin 1 and ILb1 as candidate genes underlying individual differences in susceptibility. *This research was supported in part by CDMRP grant W81XWH-17-1-047.*

PS 3060 Assessing the Differential Sensitivities of a Microelectrode Array Assay and a Neurite Outgrowth Assay for Detecting CNS Liabilities Using a Set of Neurotoxic and Seizurogenic Compounds

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Domoic acid is a neurotoxin first associated with the poisoning of 107 individuals and three subsequent deaths on Prince Edward Island in 1987 after these individuals consumed mussels containing this toxin. Due to its neurologic adverse effects, including memory loss, the illness was called amnesic shellfish poisoning. The toxin has since been attributed to the diatom, *Nitzschia pungens* f. *multiseries*, which was ingested by the mussels during normal filter feeding. In our lab, we use domoic acid as a tool compound when testing neurons on a microelectrode array (MEA) platform (Axion Biosystem's Maestro). Domoic acid completely eliminates all spontaneous spike activity when treating neurons at concentrations > 1 µM. When tested in a neurite outgrowth assay, using a high content imager (ArrayScan VTi), domoic acid does not affect cell health or neurite outgrowth when tested up to 10 µM for 72 hours. We tested various neurotoxins and seizurogenic compounds on the MEA platform and in the neurite outgrowth assay to the compare results and assay sensitivities. The antipsychotic haloperidol demonstrated seizurogenic liabilities at 3.16 µM on the MEA but had an IC50 of > 30 µM in an HCS assay. $GABA_A$ antagonist, picrotoxin, has a seizurogenic MEA profile as low as 0.2 µM but does effect cell health and neurite outgrowth at concentrations up to 10 µM. Chlorpromazine, on the other hand, has a seizurogenic profile and effects cell health and neurite outgrowth at similar concentrations (3 µM). With the results from domoic acid, haloperidol, picrotoxin, chlorpromazine and 6 additional controls compounds, we have determined that the MEA platform is more sensitive to detecting electrophysiological CNS liabilities than the neurite outgrowth assay. Alternatively, compounds which affect neurons by non-electrophysiological cytotoxic effects can be detected more sensitively than the acute MEA assay. Therefore, an effective overall strategy would be to test compounds for safety in both assays.

3061 Neurotoxicity of Diethylene Glycol in a Rat Model

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Diethylene glycol (DEG) can be found in consumer products, but can also be an adulterant in medicines by acting as counterfeit glycerin. DEG poisonings have been characterized predominately by acute kidney injury (AKI), but have also been known to affect the nervous system via delayed neurological sequelae such as decreased reflexes, face and limb weakness, or quadreparesis, as observed in a patients 2-7 days after DEG ingestion. Characterizing these poorly understood neurological symptoms of DEG poisonings in an animal model can clarify the overall toxicity and make mechanistic connections between the kidney injury and neuropathy. Male Wistar-Han rats were administered by oral gavage a water control or doses of 4 - 6 g/kg DEG every 12 or 24 h and monitored in metabolic housing for up to 7 days. Urine was collected every 12 h and endpoint blood and cerebrospinal fluid (CSF) were collected for renal plasma biomarkers and total protein estimation, respectively. Motor function tests were conducted before and after treatment. Kidney, brain, and spinal cord tissue were harvested after euthanasia for later pathology analysis. Of the 29 rats that were treated with DEG, 8 developed AKI with confirmation by renal plasma biomarkers. AKI primarily occurred after doses that were administered every 12 h. There was a marked increase in renal DGA accumulation in rats that developed AKI, compared to rats without AKI, confirming the role of DGA in the nephrotoxicity. The level of DEG in all treated animals stayed in the same range (15-25 mg/mL), with no obvious differences between rats with and without AKI. The total protein content of CSF in rats with AKI was significantly higher than controls and rats without AKI, indicating the first evidence of nervous system damage from DEG treatment in an animal model. Significant decreases in grip strength as well as in locomotor and rearing activity were observed in rats with AKI compared to controls and to rats without AKI. Initial pilot studies did not show dramatic changes in myelination (Luxol fast blue) in animals with elevated CSF protein, compared to controls or to unaffected but treated animals. The observation of increased CSF protein and motor function deficits, only in rats with AKI, indicate the development of neurotoxicity in this subacute animal model and strongly suggest that kidney injury needs to occur before neurological symptoms are observed. *Funding Source: American Chemistry Council and NIH (R15 ES029704).*

3062 Environmental Polychlorinated Biphenyls Mixtures Inhibit the Dopamine Transporter

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Dopamine is a monoamine transmitter that contributes to the motor and reward system in in the body. When neurons experience a change in dopamine availability it can cause symptoms of addiction, attention deficit hyperactivity disorder (ADHD), and Parkinson's disease. Polychlorinated biphenyls (PCBs) congeners are man-made environmental pollutants that disrupt the dopamine transporter (DAT) that is responsible for dopamine uptake from the synaptic cleft. When DAT is altered it may lead to altered cognitive proficiency. To date 46 non-dioxin like (NDL) PCB congeners have been found to alter DAT activity and include those highly chlorinated in the ortho position. NDL PCBs are present in the environment as mixtures; however, currently how these mixtures contribute to altered activity is currently unknown. The focus of this study is to examine the activity of PCBs in binary and complex mixtures towards DAT. I will use radioligand binding assay in female rat synaptosomes, with [3H] WIN- 35, 428 in the presence or absence of single PCBs or the mixture there of. Preliminary data confirms the inhibitory activity of NDL PCB congener PCB 95. The binding assay inhibitory concentrations (IC50) from this work and that of others will then be used to develop and apply a neurotoxic equivalency scheme (NEQ) to published PCB mixtures concentration to predict DAT activity. Preliminary applications of a DAT NEQ based on the highly potent PCB 110, found that the serum of children serum in East Chicago had a NEQ of 10.73 nanograms per gram. This NEQ represents a measurement of neurotoxicity relative to PCB 110. The ability of NDL PCBs to induce DAT inhibition and alter dopamine concentrations can aid in addressing its harmful persistent neural activity during neurodevelopment and its effect on initiating neurological disorders especially Parkinson's disease. *Supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM071638.*

3063 A Chemically Defined Hydrogel Substrate Promotes Accelerated Maturation and Neurite Extension of Cortical Glutamatergic and Motor Neurons for High-Throughput Screening

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Human neural cells manufactured from patient-derived induced pluripotent stem cells (iPSCs) hold great promise for modeling neurodevelopmental disorders, discovering precision therapies, and screening for potential risks from environmental toxins. However, many neurological phenotypes arise in mature neurons, and human iPSC-derived neurons can require extensive time in culture (1-3 months) to reach full maturity. These lengthy cultures slow the discovery process and are costly due to labor and reagent requirements. We hypothesized that optimizing culture substrate properties through the use of tunable synthetic matrices would improve maturation. Currently, neurons are cultured on a variety of substrates including charged polymers (poly-lysines or poly-ornithines) or animal-derived matrices. Using JMP™ software, we employed Design of Experiment (DOE) methodology utilizing Box-Behnken response surface modeling to screen for synthetic polyethylene glycol-based (PEG) hydrogel formulations that promoted viability, cell adhesion, desired morphology, and accelerated maturation of cortical glutamatergic neurons. In the experimental design, we varied PEG concentrations, crosslinkers and cell adhesion peptide composition and concentrations. To facilitate the quantitative DOE analysis, we utilized neurons derived from a human iPSC reporter line with a fusion protein comprising nanoluciferase (Nluc, Promega) and synaptophysin (SYP), a synaptic vesicle glycoprotein that is expressed in virtually all mature neurons and acts as a marker for quantification of synapses. We identified hydrogel formulations that 1) support cortical glutamatergic neuron adhesion as scored morphologically and assessed quantitatively via cellular ATP (Cell Titer Glo 2.0, Promega) and 2) accelerate maturation, as demonstrated through a time-course of synaptophysin expression. Finally, these hydrogel formulations supported over two-fold increases in neurite length over a poly-D-lysine substrate. When incorporated into neuronal high-throughput screening efforts, the identified hydrogel substrates will improve overall outcomes and decrease the culture time required to reach the necessary maturation state.

3064 Chronic Glucocorticoid Exposure Primes the Neuroinflammatory Response to Nerve Agent Sarin

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Chronic exposure to the glucocorticoid corticosterone (CORT), at levels associated with high physiological stress, can exacerbate CNS proinflammatory responses to neurotoxic insults in animal models. Persistent sickness behavior, a prominent component of Gulf War Illness (GWI), is associated with neuroinflammation. Veterans of the 1991 GW were exposed to the stresses of war, being prophylactically treated with the reversible acetylcholinesterase (AChE) inhibitor pyridostigmine (PB), organophosphate pesticides chlorpyrifos (CPO) and dichlorvos (DDVP) and potentially the nerve agent sarin. We have previously shown CORT exacerbation of the neuroinflammatory response to CPO, DDVP, and the sarin surrogate diisopropyl fluorophosphate (DFP). Here, we confirm that sarin exposure also causes a neuroinflammatory response that is exacerbated by chronic CORT pretreatment. CORT (200 ug/mL in 0.6% EtOH) was given in the drinking water for 1 week prior to sarin administration at an LD20 dose (0.1 mg/kg, s.c.) on day 8. Animals were euthanized at 6 hours and brains were dissected and then frozen for RNA and protein analysis. RNAseq analysis of cortex revealed 1535 genes that were significantly up-regulated in the CORT+sarin group. Of these, 211 were significantly greater than sarin alone. These 211 genes were interrogated with DAVID to find GO terms which included cytokine production, MAP kinase phosphatase activity, and cytokine binding. Kegg pathways include: cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, MAPK signaling pathway, and hematopoietic cell lineage. The neuroinflammatory response was further confirmed with elevated pSTAT3 protein by ELISA and elevated neuroinflammatory cytokines and chemokines mRNA (TNFα, IL6, CCL2, IL1β, LIF, and OSM) by qPCR. Together these findings confirm those we have previously shown with sarin surrogate, DFP and provide additional support for the hypothesis that GWI is a chronic, stressor-primed, neuroinflammatory condition potentially instigated by the combined exposures to stressors and irreversible AChE inhibitors.

3065 Microscopic Analysis of the Intracellular Distribution of the Aryl Hydrocarbon Receptor at the Single Neuron Level *In Vivo*

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The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is present in the cell cytoplasm and plays an essential role in the induction of various dioxin toxicities, including neurotoxicity. Once activated by a ligand such as dioxin, the AhR-dioxin complex translocates from the cytoplasm into the nucleus, and induces its downstream signaling cascade. This signaling event suggests that the intracellular dynamics of AhR is involved in dioxin toxicities. However, the dioxin-induced nuclear translocation of AhR *in vivo* remains to be unclear. Thus, this study aimed to identify AhR-expressing neurons in the mouse brain by immunofluorescent staining, and examine dioxin-activated AhR distribution in neurons. Mice were administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at a dose of 20 µg/kg b.w. or vehicle (control group). To identify AhR-rich-brain regions, we utilized the Allen Brain Atlas, an *in situ* hybridization database for numerous transcripts in the mouse brain. A distinct *AhR* transcript signal was observed in the locus coeruleus (LC) of the developing and adult mouse brains. The LC consists of noradrenergic (NA) neurons expressing tyrosine hydroxylase (TH); therefore, we examined AhR expression in NA neurons (LC-NA neurons) in the brains of 5-, 7-, and 14-day-old mice, using anti-AhR and TH antibodies. Microscopic analysis revealed that almost all of the TH-positive cells in the LC were positive for AhR, and that the immunostaining intensity of AhR in the nucleus of LC-NA neurons remained unaltered during development. AhR was not detected in LC-NA neurons of *AhR*-null mice (a kind gift from Prof. Y. Fujii-Kuriyama, then at University of Tsukuba) by immunostaining, confirming the specificity of the anti-AhR antibody. Furthermore, we analyzed the distribution of AhR in LC-NA neurons of TCDD-exposed adult mice (12-weeks old), and revealed a significant increase in nuclear AhR in LC-NA neurons in the TCDD group compared to that in the control group. In conclusion, this study is the first to demonstrate the ligand-induced nuclear translocation of AhR at the single cell level *in vivo*. Our findings provide a new approach to reveal molecular mechanisms of neurotoxicity induced by AhR agonists.

3066 Effects of N-Acetylcysteine on Mouse Primary Spinal Cord Astrocyte During MeHg-Induced Toxicity

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A low concentration of methylmercury (MeHg) has been reported to be toxic to motor neurons (MNs) derived from mouse spinal cord, human iPSC and brainstem slices of humanized transgenic SOD1 G93A mice. Mechanisms of MeHg toxicity on MNs include dysregulation of intracellular calcium homeostasis, glutamate transmission, and redox homeostasis. Astrocytes (AST) play important roles in the maintenance of glutamate and redox homeostasis in neurons. The antioxidant N-acetyl cysteine amide (NACA) provided protection against MeHg-induced SCA degeneration to primary cultures of spinal cord astrocytes (SCA). In this study, we examined perturbation by MeHg of the Nrf2 pathway in SCA by determining the expression of antioxidant genes underlying Nrf2, and the role of NAC in the protection of SCA against MeHg toxicity. NAC caused a concentration-dependent protection indicated by the reduction of cell viability. SCA were pretreated with 0.1mM, 1.0mM, or 10mM NAC 2h before exposure to 5µM MeHg or cotreated continually with these concentrations of NAC with MeHg. At 0.1 mM, NAC lost its efficacy at protection against MeHg-induced SCA degeneration at 30h of exposure, while 1mM and 10mM NAC maintained their protection across 150h of 5µM MeHg exposure. The 1mM NAC was sufficient to protect SCA; the level of protection at this concentration did not differ from that at 10mM NAC or vehicle treatment level. At 18h of MeHg exposure, MeHg induced the reduction of Keap1, Gclc, Gpx1, Gpx4, and Txnrd1 mRNAs but not Nrf2 mRNA expression. Application of 1mM NAC before, or cotreatment with MeHg, prevented reductions of the antioxidant mRNAs which were maintained at the same levels of the vehicle and NAC treatment alone. The mechanism by which NAC causes protection against MeHg could be partly to it activating Nrf2 since NAC treatment alone induced the increase of Nrf2 mRNA expression. *This research was supported by NIH grant R01ES024064, and R25NS090989- ENDURE Program.*

3067 Potential Toxicity Induced by Exercise Mimetics AICAR and GW501516 on Voluntary Exercise Capacity of Ischemic Mice

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Despite stroke being the leading cause of permanent disability in the world, no FDA approved pharmacological treatment is available for promoting functional recovery from stroke related disabilities. Exercise mimetics are an emerging class of pharmacological agents deemed to possess the effects of physical exercise on the body. It is believed, such agents may be used to replace physical exercise in patients with impaired motor function. In this study, we hypothesized that the combination therapy of AICAR and GW501516, two most studies exercise mimetics, could promote motor function recovery after ischemic stroke. However, potential toxicity of this combination therapy on exercise capacity of mice was observed when AICAR+GW was given along with voluntary running exercise after ischemic stroke in mice. For this study, we used photothrombotic stroke model to induce ischemic stroke in CD1 male mice. For pharmacological treatment, AICAR (5mg/kg, IP) and GW501516 (500 mg/kg, SC) were given every other day starting from 24 hours post-stroke and continued until 9 days post-stroke. The mice started voluntary exercise with running wheel from 7 days post-stroke and continued until 20 days post-stroke. We measured the amount of exercise per mouse per session with the help of a digital counter attached to each running device. Additionally, to assess motor recovery gridwalk test was performed on 3 days pre-stroke and 1, 7, 14, 21days post-stroke. Our results indicate, the drug treated mice group was running consistently less compared to the vehicle treated group. In fact, at the later stage of the experiment, the vehicle treated group was running on average around 10,000 meters per day however, the drug treated group was running half of that amount, around 5,000 meters per day. These running discrepancy indicated a potential drug induced toxicity in the drug treated group. Additionally, our active running duration data indicate, the AICAR+GW501516 treated group was actively running almost half of time of vehicle treated group with an average value of around 2 hours and 4 hours for drug and vehicle treated group, respectively. In summary, we observed a reduced voluntary exercise capacity in terms of running distance and active running duration after AICAR+GW501516 treatment in ischemic mice. However, the mechanism of this potential toxicity has not been elucidated and further study is needed to elucidate the molecular mechanism of this observed toxicity.

3068 Applicability of hiPSC-Derived Neuronal Co-cultures and Rat Primary Cortical Cultures for *In Vitro* Seizure Liability Assessment Using Micro-Electrode Array (MEA) Recordings

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Seizures are life threatening events in which neurons are hyper-excitable and fire in a hyper-synchronised manner. Drugs targeting the central nervous system pose a high risk for seizures. The potential of compounds to cause seizures is not investigated until late in the drug developmental process, meaning that once it is discovered, a lot of time and money has been invested. On top of that, current rodent based models are not always able to predict human seizurogenic compounds. There is thus a clear need for an *in vitro* screening model for seizure liability assessment, preferably using human cells to circumvent inter-species differences and to limit the number of test animals needed. We hypothesized that such human models outperform rodent models. The present study therefore aims to assess the suitability of hiPSC-derived neurons for seizure liability assessment in comparison to rat cortical cultures. We cultured an hiPSC-derived neuronal co-culture model and exposed it to eight known (non-)seizurogenic compounds (PTZ, amoxapine, enoxacin, amoxicillin, linopirdine, pilocarpine, CPZ and phenytoin). Using micro-electrode array (MEA) recordings we assessed the effect of these compounds on neuronal network activity. Data obtained from the hiPSC model was compared with data simultaneously obtained from rat primary cortical neurons. All compounds, except PTZ and enoxacin, increased either the spike, burst or network burst rate following acute exposure of hiPSC-derived neuronal cultures. Seizures were not detected in rat primary cortical neurons following exposure to amoxicillin and pilocarpine. When seizures were detected in both models, LOECS were comparable in the case of amoxapine and CPZ (LOEC 1 µM). For linopirdine, hiPSC-neurons were ten times more sensitive (LOEC 3 µM), whereas for phenytoin rat primary cortical neurons were more sensitive (LOEC 30 µM versus 100 µM voor hiPSC-neurons). We created chemical fingerprints that show that spike parameters are more sensitive for excitability in rat primary cortical neurons, whilst in hiPSC-derived co-cultures (network) burst parameters are more affected. Our data indicate that hiPSC-derived neuronal co-cultures are able to detect seizures in a manner comparable to rat pri-

mary cortical neurons. *This work was funded by NC3Rs (project number 50308-372160), ZonMW (project number 114027001) and the Faculty of Veterinary Medicine (Utrecht University, The Netherlands).*

3069 Important Laboratory Monitoring Parameters to Prevent Valproic Acid-Induced Toxicity in Long-Term Care Facilities

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Long-term care (LTC) facilities have experienced an increase in the utilization of valproic acid (VPA) for residents suffering behavioral and psychological symptoms of dementia (BPSD). This indication is a promising strategy for antipsychotic deflection. However, there is concern that VPA is lacking the appropriate monitoring and also may be inappropriately titrated to antiepileptic blood levels, rather than therapeutic response for this indication, leading to side effects and neurotoxicity. VPA is usually well-tolerated, but previous studies have reported serious valproic acid-induced toxicity, including hepatotoxicity, thrombocytopenia, and hyperammonemic neurotoxicity, etc. Moreover, consistent monitoring parameters at low doses for indications other than epilepsy are often not obtained. Therefore, the purposes of this study are to characterize the use of VPA in LTC facilities and demonstrate the importance of VPA monitoring parameters in prevention of toxicity. The study population consisted of 1412 elderly residents residing in 13 LTC facilities across the state of Texas. Dispensing records, VPA blood levels, ammonia levels, and platelets for all residents during the 3-month study period was filtered and analyzed. As the result, the total daily doses of VPA ranged from 125 mg to 3000 mg with the majority (56%) received 125-500 mg, followed by 29% and 15% receiving 501-1000 mg and >1001mg, respectively. Seventy-eight percent of the residents received VPA for indications other than epilepsy, with 14.6% of those residents were within antiepileptic blood levels. Regarding VPA-induced toxicity, 13.8% of residents had thrombocytopenia with the vast majority (91.3%) of these residents receiving <1250 mg VPA daily. Hyperammonemia also appears to be associated with appropriate VPA dose of 125-1500 mg daily and sub-antiepileptic VPA blood levels (< 50 mg/L). The data support that hyperammonemia and thrombocytopenia are not dose related nor proportional to the blood VPA level. The results of this study emphasize the importance of appropriate monitoring ammonia levels and platelet counts regardless of VPA total daily dose, especially when used for BPSD.

3070 Effects of Age on the Susceptibility to Neurobehavioral Toxicity following Acute Domoic Acid Exposure in a Mouse Model

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The harmful algal bloom (HAB) toxin domoic acid (DA) contaminates marine food webs in the western United States, and acts as a potent glutamate receptor agonist following ingestion. With the duration, severity, and toxicity of HAB events expected to increase in coming years, spatial and temporal windows of concern for DA exposure are widening. It is critical that we characterize risk for members of the human communities that depend on safe seafood resources. The elderly are disproportionately represented among those that have suffered from acute DA toxicosis, and new research suggests that they are at risk for chronic DA exposures. Reduced resilience in response to physiological stress is a hallmark of aging. This reduced resilience is associated with higher systemic levels of oxidative stress and inflammation, both of which are also associated with DA toxicity. Therefore it is important to identify possible increased susceptibility of the elderly to the toxic effects of DA exposure. To this end, we are conducting a dose-response study assessing convulsive and subconvulsive responses to acute administration of DA (1-2.5 mg/kg) in young (7-9 mo) and aged (24-28 mo) mice. By assessing both subconvulsive and convulsive responses we will assess sensitivity to DA across a range of exposures. Tissue concentrations of DA at the time of observed behaviors will be measured in an effort to identify possible differences in renal clearance or tissue uptake of DA. Additional markers of oxidative stress, endoplasmic reticulum stress, neuronal apoptosis, and ultrastructural damage will be assessed to identify biochemical and histological correlates of the interaction between age and DA toxicity in the mammalian brain. A comparison of dose-response curves will allow for discussion of the extent to which age may play a role in DA susceptibility.

3071 Spontaneous Incidence of Infrequent Neurological Clinical Signs across Species: A Retrospective Analysis

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The identification of possible adverse effects is crucial in the development and safety assessment of drugs in toxicology and safety pharmacology studies. Control groups allow for the discrimination of findings (for example, changes in symptoms, signs, or other morbidity) caused by the test-item treatment from outcomes caused by other factors which could be unrelated to drug treatment. Potential spontaneous clinical observations in healthy animals can confound the interpretation of test-item related effects. Characterizing these infrequent species-specific spontaneous findings can help in the interpretation of true treatment related clinical observations. The purpose of this retrospective analysis was to determine the prevalence of incidental clinical observations which could be interpreted as neurologically related in control animals. Clinical observations documented in control animals from 141 GLP-compliant toxicology studies which were conducted between 2015 and 2019 were compiled. These studies consisted of a total of 5022 control animals (both males and females) with dosing phases ranging from 1 day to 39 weeks and recovery phases ranging from no recovery to 26 weeks. All control animals across these studies were dosed with an appropriate vehicle consistent with the formulation buffer. Species evaluated consisted of Beagle dogs, Sprague-Dawley, Wistar and Athymic Nude rats, New Zealand White rabbits, Göttingen minipigs, Cynomolgus and Rhesus monkeys, and CD-1 mice. Among the clinical signs compiled which could be interpreted as neurologically-related, signs such as salivation, tremors, uncoordinated, myoclonic jerks, muscle twitches, altered muscle tone, muscle atrophy, full muscle contraction, circling, clonic convulsions, tonic convulsions, hunched back, piloerection and hypersensitivity were observed. The prevalence of these incidental observations across the different control species was analyzed which could support interpretation of treatment-related data that is confounded by neurological incidental findings.

3072 The Impact of Environmental Organochlorines on Disruption of Dopamine Homeostasis

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Disruption of dopamine homeostasis is a potential mechanistic link between an insult, such as exposure to pesticides, and injury/disease. Organochlorines present in the environment may alter dopamine metabolism and homeostasis. Dopamine is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by monoamine oxygenase. Previous work found DOPAL to be toxic, produce ROS, and be highly reactive towards proteins. Our goal is to determine the extent to which dieldrin, and polychlorinated biphenyls (PCBs) alter dopamine homeostasis, and if such disruption accounts for adverse outcomes. Specifically, PCB-52 and its human metabolite, 4-OH PCB-52, since PCB-52 has been found to be a major contributor towards PCBs in indoor and outdoor air. While these compounds are currently banned, they persist in the environment and pose a risk to human health. PCBs are associated with neurodevelopmental disorders, such as ADHD, while dieldrin has been linked to neurodegenerative disorders, including Parkinson's disease. Our hypothesis is that organochlorine environmental agents alter dopaminergic homeostasis yielding toxic outcomes. We have studied the toxicity of these compounds to PC12 and N27 cells, dopaminergic rat cell lines. Results show that 4-OH PCB 52 is toxic to dopaminergic PC12 and N27 cells at concentrations less than that of PCB-52 (less than 25μM) at 24hrs. The ability of PCB-52, and 4-OH PCB-52 to produce reactive oxygen species has been determined using fluorescent probes for ROS. Both PCB-52 and 4-OH PCB-52 increase mitochondrial and whole cell ROS in dopaminergic cell lines. However, this seems more prominent in mitochondria, and we expect similar results with dieldrin. Furthermore, we have used an aminophenylboronic acid agarose resin to select for DOPAL-modified proteins. Preliminary results show that treatment of N27 cells with DOPAL increases the amount of catechol-modified proteins present. We aim to identify proteins that are targets of DOPAL and/or indirect targets of organochlorines. To support such findings, we will determine how organochlorines alter dopamine metabolism in dopaminergic cells. This research will help determine the impact of organochlorines on the dopaminergic system, and provide insight into potential therapeutics to combat neurodegenerative and neurodevelopmental conditions.

3073 New Method for Detection of Seizure Potential Using Rat Electroencephalogram

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Seizure is an important issue during drug development. Therefore, detection of seizure potential risk is one of the most important subjects in non-clinical studies. Recently, electroencephalogram (EEG) can play a critical role in safety assessment of drugs. While EEG is widely used to the seizure assessment, the changes of EEG before seizure event are not well understood. Therefore, in this study, we aimed to develop a novel analysis method to detect seizure potential risk using rat EEG even before occurring of seizure. Adult male Sprague-Dawley rats implanted with electrodes in prefrontal cortex and hippocampus were used, and EEGs were measured for about 3 hours after treatment of 10 seizure-inducible compounds (4-aminopyridine, pentylenetetrazol, pilocarpine, picrotoxin, strychnine, isoniazid, aminophylline, bupropion, tramadol and venlafaxine) and 3 negative control compounds (acetaminophen, aspirin and amoxicillin). Animals were divided into the high and low dose groups, which showed seizure symptoms and only preceding symptoms of seizure, respectively. Then, the EEG data was classified into 6 bands [theta (5-8 Hz), alpha (8-14 Hz), beta (15-25 Hz), gamma (30-50 Hz), high gamma (70-150 Hz) and a higher band (150-200 Hz)], and 7 parameters were calculated from each band of EEG in rats, which included spike rate, burst rate, inter burst interval, burst duration, spike in a burst, peak amplitude and inter peak interval. As a result, rats showed the seizure or only preceding symptoms (twitch, straub tails, tremors etc.) after treatments, and the abnormalities of waveform was noted in high gamma band in the EEG during seizure in the high dose groups. Analyzing of the high gamma band in EEG, spike rate and burst rate increased and inter burst interval shortened during seizure but not the negative control groups. Moreover, changes in same parameters were also detected in animals which had not shown seizure, suggesting that these parameters might be related to seizure. In conclusion, we revealed the novel analysis method that can be detected seizure potential risk even at the low dose groups that doesn't cause seizure, and EEG might be useful tool to detect a preceding biomarker for seizure in non-clinical studies.

3074 Acute Cadmium Treatment Induces Deficits in Metal Homeostasis and Autophagy via Impairing Metal Transporter Systems and Energy Homeostasis in Huntington's Disease Striatal Cells

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Huntington's disease (HD) is functionally linked to environmental factors including metal dyshomeostasis and cigarette use. Interestingly, one of the most abundant heavy metals in cigarettes is cadmium (Cd), which also accumulates in the corpus striatum within the basal ganglia and causes neurotoxicity upon overexposure. We have previously reported that mutant HD protein coupled with acute Cd exposure activates protein kinase C delta dependent oxidative stress signaling mechanisms to cause neurotoxicity and cell loss in a mouse striatal cell line model of HD. To investigate the basis for the synergistic interaction between mutant HD and Cd, we hypothesized that mutant HD would alter metal transport, mitochondrial bioenergetics and protein degradation mechanisms with Cd exposure. Inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed that the mutant HD genotype is associated with increased accumulation of Cd upon Cd exposure for 48h. Further, we did not observe a significant change in the levels of iron, manganese, zinc, cobalt, copper, and nickel in wild-type and mutant HD striatal cells following Cd exposure. Examination of metal transporter system implicated in Cd transport showed a decrease in the expression of divalent metal transporter 1 (DMT1) protein in mutant HD striatal cells with Cd exposure. Additionally, pretreatment with zinc, manganese, and iron as well as exogenous antioxidants rescued the Cd-induced neurotoxicity in the mutant HD striatal cells. We observed a significant decrease in energy homeostasis and increased proteasomal activity in mutant HD striatal cells as early as 6 hours with Cd exposure. Finally, acute Cd exposure did not affect the expression of huntingtin protein but significantly regulated the expression of autophagy related proteins LC3-II, ATG5, and Beclin-1. Together, these findings suggest that mutant HD exhibit greater neurotoxic properties when in tandem with Cd exposure to cause neurotoxicity via impairing metal transport and energy homeostasis as well as regulating autophagy and proteasomal degradation signaling mechanisms.

3075 Aflatoxin-Induced Lipotoxic and Non-lipotoxic Dyslipidemia in Lymphocytes and Brain of Female Albino Rats

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Aflatoxins are a group of naturally occurring mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, which typically affect grains and nuts. Aflatoxin B1 intake has been associated with immunotoxic and neurotoxic end-points in man and animals though the mechanism(s) involved is not well established. Studies have shown that dyslipidemia is an early pathological event in various diseases. This study therefore investigated the effects of aflatoxin B1 on lipid metabolism of rats. Thirty six (36) female albino rats were exposed to aflatoxin B1 (20 ppb and 40 ppb) for 2, 4 and 6 weeks. Control rats (n=18) received normal feed for the same period. At the end of aflatoxin B1 administration, lipid profiles were determined in the lymphocytes and brain of the animals. Lipotoxic and non lipotoxic dyslipidemia were observed as a result of aflatoxicosis. In the lymphocyte, aflatoxin B1 induced a dose-dependent hypercholesterolemia (42 and 31%) at 4 and 6 weeks respectively, whereas the 20 ppb dose induced hypocholesterolemia at 2 weeks. Lymphocyte triacylglycerol and phospholipid were decreased at 2 weeks, but increased at 4 and 6 weeks respectively. While brain cholesterol decreased throughout, brain triacylglycerol increased at 2 weeks, but decreased at 4 and 6 weeks with 40ppb dose of aflatoxin B1. Phospholipidosis was the hallmark of aflatoxin B1 exposure in the brain at 4 and 6 weeks. A 35% inhibition in brain HMG CoA reductase activity was observed at 4 weeks whereas the inhibition amounted to 15% by 6 weeks of aflatoxin B1 exposure. The findings of this study indicate that the immunological and neurological effects induced by aflatoxicosis may be mediated in part by the lipotoxic and non-lipotoxic dyslipidemia.

3076 Inhibition of Synaptic Plasticity and Brain/Blood Levels of 1-Bromopropane in Rats during Inhalation

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1-Bromopropane (1-BP) is an organic solvent used in degreasing agents and spray adhesives. Several studies have reported that 1-BP exerts toxic effects on the central nervous system, manifesting symptoms such as memory problems, in occupationally exposed populations. The adverse effects on memory are due to changes in synaptic efficacy in the brain. However, the adverse effects on synaptic plasticity and 1-BP levels in the brain that change synaptic plasticity have not been reported. We previously reported that a 2-compartment model is efficient for analyzing 1-BP concentration in the brain and blood by inhalation (2017 SOT). Therefore, the blood concentration of 1-BP that causes adverse effects on synaptic plasticity can be estimated in rats. Here, we aimed to determine the 1-BP blood level that inhibited long-term potentiation (LTP), which is induced in hippocampal slices from rats. LTP was induced by high-frequency stimulations in the hippocampal CA1 and dentate gyrus of hippocampal slices prepared from naive rats. The slices were directly perfused with 1-BP solution (0.007-6.6 mM in the slice chamber) for 5 min immediately before stimulation. The other rats were exposed to 1-BP at concentrations of 50, 200, 700, and 1500 ppm for 120 min. Blood samples were collected over time via cardiac catheterization, and brains were obtained by decapitation at 15, 30, 60, and 120 min of inhalation. The 1-BP concentration was determined using head-space gas chromatography. Subsequently, the 2-compartment model was applied to the data analysis. Our results showed that the 1-BP level that inhibits LTP in the CA1 was equal to or higher than 0.06 mM, although an inhibitory tendency was observed at the lowest concentration of 0.007 mM. The level of 1-BP in the brain exceeded the inhibition level within 15 min at 200 ppm. The ratio of blood-to-brain and brain-to-blood transfer rate constants, calculated from the 2-compartment model, was less than 1 at 50 ppm and increased as the inhalation concentration increased. Our study demonstrated that 1-BP inhibits synaptic plasticity, suggesting that the blood level of 1-BP is a useful index of adverse effects on synaptic plasticity.

3077 Endogenous and Environmental Stimuli Affect Sab-Mediated Cellular Vulnerability to Neurotoxic Exposures

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Perturbations in mitochondria-cell signaling drive organelle dysfunction aging and neurodegenerative diseases. Scaffold proteins dictate the outcomes of signaling events by directing the localization, organization, and abundance of signaling proteins. Our previous work demonstrates that the increasing concentrations of the outer mitochondrial membrane (OMM) scaffold Sab renders cells vulnerable to toxic insults in a c-Jun N-terminal kinase (JNK)-dependent manner, and inhibiting Sab-mediated signal protects against chemical exposures. Because, Sab levels are elevated in vulnerable brain regions, such as the hippocampus and substantia nigra, identifying stimuli that affect Sab levels could represent mechanisms of neurodegenerative pathogenesis or uncover neuroprotective strategies. Therefore, we developed an in-cell western (ICW) for Sab to evaluate if cellular and environmental stimuli alter Sab concentrations through endogenous mechanisms. The ICW performed in HEK-293 cells (Z′ = 0.71) and validated using Sab-specific shRNAs and overexpression. A small-scale screen revealed that sub-chronic exposures to mitochondrial toxins (rotenone and antimycin) and tumor necrosis factor α (TNF-α) increased Sab levels, while antioxidants (quercetin and N-acetylcysteine) reduced Sab concentrations. Signaling pathways also affected Sab concentrations. Specifically, protein kinase A (PKA) inhibitors increased Sab levels on mitochondria, and PKA activators caused a decrease in Sab abundance in a kinase-and organelle-dependent manner. Agonists of estrogen signaling were also found to decrease Sab levels, while antagonists were found to increase Sab concentrations. Finally, we discerned that benzo[a]pyrene, a byproduct of incomplete combustion, increased Sab concentrations on mitochondria, an effect that was partly diminished in JNK inhibitor (5μM SR-3306). To determine if these chemical-induced changes in Sab concentrations had a physiological impact on cells, we pretreated HEK-293 cells with either antimycin A or estradiol. Antimycin A pretreatment increased apoptotic priming and rendered cells vulnerable to chemical insults, which was reversed by silencing Sab. Alternatively, estradiol treatment increased anti-apoptotic protein levels on mitochondria and enhanced cellular resiliency to chemical exposures. These effects were mitigated by overexpression of Sab. Our findings demonstrate that Sab concentrations are influenced by the intracellular and extracellular environments, and these endogenous and exogenous cues may provide insight into the vulnerability of discrete neuronal populations.

3078 Study on Biomarkers Exposed to 1-Bromopropane

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1-Bromopropane (1-BP) has been used in many industries with significant adverse effects on nervous system. Animal tests and occupational survey were performed to explore the exposure and effect biomarkers of neurotoxicity induced by 1-BP. Methods: 1. *In vivo* study: Male Wistar rats were exposed to 0, 500 and 1000 ppm 1-BP (18/group), 6 h/day for 21 consecutive days. Six rats of each group were euthanized on the 7th, 14th and 21th days. The brain tissues, urine and serum were collected. Histopathological examination was applied. 2. Epidemiological survey: The exposure and control groups were studied with 71 workers in each group. Serum and urine were collected after work. Concentrations of 1-BP in the work sites and individuals were tested. 3. Changing of NSE, S-100β and COX-2 in the cerebral cortex of rats and the serum of exposed workers were measured respectively, changes of 1-BP and its metabolite AcPrCys in the urine were detected. 1. *In vivo* study: Purkinje cell atrophy, lumbar gray matter vacuolar degeneration, tibiofibular nerve fibers swelling and thickening were tested at 1000ppm on the 21thday. cNSE and cS-100β at 1000ppm, sNSE at 500ppm were increased significantly at all checkpoints. sS-100β at 1000ppm on the 21th days was increased significantly. Compared with the control, cCOX-2 at 500 and 1000ppm was increased greatly as well as at 500ppm on the 14th and 21th days compared with the 1000ppm group. sCOX was increased greatly on the 14th days at 500ppm, and on the 7th and 14th days at 1000ppm. A correlation between the changes of COX-2 in the cerebral cortex and serum was found, and 1-BP and AcPrCys were detected in the urine, there was a correlation between the changes of sNSE and sCOX-2 and AcPrCys in the urine at 500ppm. 2. Epidemiological survey: The concentration of 1-BP in the use enterprises was generally higher than that of the production enterprises. 1-BP was detected in some urine samples and AcPrCys was detected in all urine samples of exposed workers. Correlation of AcPrCys and exposure concentration was detected. 1. Subacute nerve injury was induced by 1-BP in rats under the conditions of this test. Concentrations correlation of COX-2 between cerebral cortex and serum was found. 2. The prototypes and the metabolites (AcPrCys) of 1-BP were detected in urine in exposure groups, and a good correlation between AcPrCys and exposure concentration was found. Results indicated that AcPrCys was more sensitive than the prototype as the 1-BP exposure biomarker.

3079 Impact of N-Methyl-D-Aspartate Receptor and Complement System on Neuronal Death Induced by High Glucose

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In hyperglycemia glucotoxicity-induced insulin resistance is one of the important causes of oxidative stress leading to neuronal death and dementia in diabetic patients. SH-SY5Y human neuroblastoma cell line can constitute an *in vitro* model to analyze complement biosynthesis by human neurons. In this study, following the high glucose exposure, the effect of neuronal nitric oxide (NOx), oxidative stress coefficient, glutamate, methylglyoxal (MGO), glucose transporter3 (GLUT3), complement1 (C1q), C3a and complement regulatory protein (CD59) levels on cell viability were evaluated at first 48-hour period in SH-SY5Y cells. Kynurenic acid (KynA), a unique N-methyl-D-aspartate receptor (NMDAR) antagonist, was used to determine whether excitatory glutamatergic neurotransmission via NMDAR leads to neuronal death in the presence of high glucose and insulin. Excessive oxidative stress, and glutamate toxicity in neurons caused by high glucose and insulin exposure, was reduced with the addition of KynA. The increase in MGO was thought to be due to the increase in GLUT3 activity and the decrease in glutamate toxicity was due to the blockade of NMDAR. Both CD59 and C3a showed parallel alterations with C1q in all experimental conditions, where the presence of glucose was mandatory. The constant variable between C1q and CD59 or C3a was calculated as 0.8. Insulin resistance decreased C1q and CD59 levels, while KynA improved insulin resistance and significantly increased C1q and CD59 levels. This study revealed that high glucose-related neuronal death due to oxidative stress and insulin resistance could be significantly improved by NMDAR antagonist, KynA. However, C1q, CD59 or C3a did not contribute the membrane attack complex formation and subsequent neuronal death in SH-SY5Y neurons, which were exposed to high glucose and insulin. *This study was supported by The Scientific and Technological Research Council of Turkey, 214S112.*

3080 Optimizing Neurotoxic Hazard Characterization of New Psychoactive Substances (NPS): Assessing Prolonged Exposure and Reversibility of Effects on Neuronal Activity Using Microelectrode Array Recordings

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New psychoactive substances (NPS) use is linked to numerous emergency department visits and deaths. However, hazard characterization of NPS is limited and often based on case studies and acute measurements on single targets, like the monoamine transporters. As this poorly relates to the human *in vivo* situation, we hypothesized that integrated measurements of neuronal activity that consider the human exposure duration and the reversibility of effects could increase the translatability and improve NPS hazard characterization. To that aim, we used rat cortical cultures grown on multi well microelectrode arrays (mwMEAs) to assess changes in neuronal activity after acute (30 min) and prolonged (5 h) exposure to 11 illicit drugs and NPS (MDMA, PMMA, methamphetamine, methylone, MDPV, α-PVP, 2C-B, 25B-NBOMe, BZP, TFMPP and cocaine). In addition, the reversibility of effects was tested following a 19 h washout period. All drugs concentration-dependently decreased neuronal activity during acute and prolonged exposure. The increase in exposure duration did not exacerbate neurotoxicity for the drugs tested. Following washout, drug- and concentration-dependent recovery was seen for all tested drugs. Following exposure to the highest concentrations of MDPV, 2C-B, 25B-NBOMe and TFMPP no recovery was seen, while exposure to methamphetamine, cocaine and BZP was fully reversible. All other drugs showed partial recovery at the highest concentration measured. Following washout, low concentrations of methylone and 2C-B caused an increase in neuronal activity compared to control. When comparing effect concentrations following washout to human concentrations, 3 out of 11 drugs were identified as long-acting substances. These long-acting substances could not be identified based on drug class, chemical structure or potency during acute exposure measurements. Hazard characterization of emerging NPS should

thus also include prolonged exposure and recovery measurements, in addition to acute exposure measurements for potency screening, to mimic human exposure as best as possible.

3081 Diglycolic Acid, the Toxic Metabolite of Diethylene Glycol, Induces Apoptosis with Secondary Necrosis in SH-SY5Y Neuronal Cells *In Vitro*

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Diethylene glycol (DEG) is an industrial solvent found in a variety of consumer products. When accidentally or intentionally ingested, toxicity manifests as severe nephrotoxicity, hepatotoxicity, and late-stage neurotoxicity. DEG metabolite-induced nephrotoxicity and hepatotoxicity have been well characterized; however, the metabolite responsible for the neurotoxicity has yet to be elucidated. SH-SY5Y cells were treated with increasing concentrations of DEG metabolites: diglycolic acid (DGA) or 2-hydroxyethoxyacetic acid (2-HEAA) for 24h or 48h. Cell death characterization was determined by flow cytometric analysis, lactate dehydrogenase (LDH) release, ethidium homodimer (EtHD) uptake, caspase-3 activation, and oligonucleosome formation. Supraphysiological 2-HEAA exposure (200 mmol/L) was the only concentration shown to induce significant necrosis. However, following DGA (6.25, 25, 50, and 100 mmol/L) exposure at both time points, SH-SY5Y cells showed significant concentration-dependent LDH release (r^2 = 0.929 at 24 h, 0.720 at 48 h) and EtHD uptake (r^2 = 0.826 at 24 h, 0.644 at 48 h) compared to controls, indicating necrosis. DGA-treated SH-SY5Y cells also showed significant dose-dependent oligonucleosome formation (r^2 = 0.841 at 24 h, 0.921 at 48 h) and caspase-3 activation (r^2 0.848 at 24h, 0.859 at 48h), but only at the 50 mmol/L concentration. Interestingly, cells exposed to DGA for 5 days showed significant EtHD uptake at concentrations as low as 15 mmol/L, indicating exposure time as an important factor in neuronal cells exposed to low, physiologically relevant DGA concentrations. Taken together, these results indicate that acute DGA treatment, not 2-HEAA, causes apoptosis followed by secondary necrosis *in vitro* at multiple time points.

3082 Correlation of Electrophysiological Parameters with Behavioral Performance in an Animal Model Using Aluminum Exposures

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We have incorporated electrophysiology as part of our neurotoxicity assessment repertoire. Specifically, we have used microelectrode arrays to effectively screen the effects of various environmental hazards and stressors on neuronal function, especially on hippocampal neurons. Electrophysiology provides quantitative assessments of synaptic transmission efficiency, synaptic plasticity, and spontaneous activity as measured by input-output (IO) relationship, short or long term potentiation (LTP), and spontaneous non-evoked activity, respectively. However, the physiological significance of changes in these electrophysiological (EP) parameters remains unclear. The overall objective of our study is to establish a correlation analysis between EP recordings and behavioral performance. We are using aluminum chloride ($AlCl_3$) exposures because they are known to induce memory loss and other cognitive deficits. Rats were exposed to $AlCl_3$ via intraperitoneal injection (IP) over 7 days at a concentration of 0 (control), 50 (mid), or 100 (high) mg/kg body weight. A separate set of rats were exposed via gavage daily for 6 weeks at the same doses. Transient decrease in motor activity was observed in the IP group, whereas impaired cognition was observed in the gavage group. In the IP group, we saw a reduction in the average IO response, enhancement in the average paired pulse facilitation (PPF), and a reduction in LTP of the mid and high dosed animals. In the gavage group, we saw an enhancement in the average IO response, enhancement in the average PPF, and reduction in LTP of the mid and high dosed animals. Small but statistically significant reduction in glutamate and acetylcholinesterase levels in the brain were observed in the IP group. Increases in plasma levels of cytokines were observed for both IP and gavage groups. Preliminary regression analysis yielded some statistically significant correlations among EP, biochemical and behavioral measurements, and thus support the hypothesis that EP recordings in conjunction with biochemical measurements can be indicative of behavioral outcome.

3083 Effects of Acute and Chronic Developmental Exposure to Bisphenol A (BPA) on Development and Function of Neuronal Networks Measured Using Microelectrode Array (MEA) Recordings in Rat Primary Cortical Cultures *In Vitro*

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Human epidemiological and animal *in vivo* data indicate an association between pre- and postnatal exposure to endocrine disruptive compounds (EDCs) and impaired neurodevelopment. Considering that only a fraction of the large number of EDCs has been studied for adverse neurodevelopment effects, efficient *in vitro* models are required to timely investigate the neurotoxic potential of EDCs. To date, however, developmental neurotoxicity of EDCs has hardly been studied *in vitro*. This study therefore aimed to assess EDC-induced (developmental) neurotoxicity using bisphenol A (BPA) as a reference EDC. To that aim, we used primary rat cortical cultures grown on microelectrode arrays (MEA) as *in vitro* model to investigate the effects of acute and developmental exposure on neuronal network function and formation. Cortical cultures were acutely exposed for 30 min to BPA (0.001-100 µM) on Day *in vitro* (DIV) 10 to assess effects on network formation. Additionally, cultures were developmentally exposed to BPA from DIV 4-21 to assess effects on network formation. Our data demonstrate that acute exposure of rat cortical cultures to BPA induced a concentration-dependent inhibition of neuronal activity from 1 µM onwards, with complete cessation of activity at 100 µM. Similarly, developmental exposure to the high concentration of BPA strongly inhibited development of neuronal activity up to DIV15. Surprisingly, after DIV15 neuronal activity recovered to control levels. These data demonstrate that acute BPA exposure inhibits neuronal activity, in line with its reported inhibitory effect on voltage-gated calcium channels. The recovery of neuronal activity after prolonged developmental BPA exposure suggests the involvement of adaptive mechanisms that may attenuate BPA-induced developmental neurotoxicity. *This project was funded by the EU-H2020 program (grant agreement No 825759).*

3084 Short- and Long-Term Behavioral Effects following Embryonic Exposure to Environmental Pollutants in Zebrafish

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Exposure to environmental pollutants during development is associated with adverse health effects throughout life. The developing brain is especially sensitive to such alterations and even very low levels of exposure to chemicals can cause persistent disorders in the cognitive function and in behavior. The zebrafish model is considered advantageous due to its low cost, small size, rapid development, and homology with mammals. The locomotor activity of zebrafish larvae can be assessed to evaluate the short-term effects of potential neurotoxic agents. In adults, more complex behaviors can be assessed and a longer exposure can be achieved which offers the possibility to evaluate the persistence or retard in the behavior caused by potential pollutants. In this study, the short and long-term behavioral effects of four environmental pollutants were assessed in zebrafish larvae and adults following embryonic exposure. 3-5 hours post fertilization (hpf) embryos were incubated in the media with three flame retardants: Triphenyl phosphate (TPP), Isopropylated phenyl phosphate (IPP) and 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47). The pesticide Chlorpyrifos (CPF) was used as positive control. Embryos were allowed to grow until 5 dpf for short-term effect or until adulthood for long-term effect. The lowest effective level (LEL) from the Locomotor Activity assay performed at 5 dpf was used as the highest concentration evaluated in the adult behavioral assessment. Three tests were performed to assess behavior in adults: the Open Field Test to evaluate general locomotor activity and two further tests to study anxiety-like / fear-related behavior: the Novel Tank Diving Test (NTT) to assess geotaxis (initial diving response) and the Light-Dark Preference Test (L/D) to assess scototaxis (preference for the dark area). The results showed that an anxiolytic-like behavior was induced by CPF and IPP, while BDE-47 provoked an anxiogenic-like effect. TPP treated fish showed opposite behaviors at different concentrations. Interestingly, long-term behavioral effects were induced at concentrations where no effect was detected in larvae stage. Therefore, zebrafish can be used as a sensitive and cost-effective model to evaluate the short and long-term neurobehavioral effects of potential environmental pollutants.

3085 Deletion of NRF2 Enhances Susceptibility to Neurotoxic Effects of Acrylamide in Mice

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Acrylamide (ACR) is an electrophile which has been used extensively in industry and is also formed unintentionally in food substances cooked or processed at high temperatures, such as potato chips or coffee through Maillard reaction. Acrylamide has been recognized as a potent neurotoxin which is known to cause neuropathy or encephalopathy in humans and experimental animals. As a measure of protection against neurotoxicity, the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) has been identified to be a master regulator of the cellular defense system which activates antioxidant and cytoprotective genes. However, knowledge about the exact mechanistic roles of NRF2 in ACR-induced neurotoxicity remains poorly understood. This study therefore sought to investigate and clarify the roles of NRF2 in attenuating ACR-induced neurotoxicity. Thirty-six male Nrf2-knockout (Nrf2-KO) mice from the C57BL/6JJcl background, aged 10-weeks together with their age and sex-matched wild-type (WT) counterparts were each divided into three groups of twelve and daily exposed to ACR at 0, 67 or 200 ppm in drinking water for 28 days. Following exposure of mice to ACR, Landing Foot spread test, an assessment of motor function and a major endpoint marker of neurotoxicity as well as immunohistochemistry for noradrenaline transporter (NAT) antibody in the dorsal and ventral medial prefrontal cortex were performed. NRF2-KO mice showed exacerbated impairment of motor functions evidenced by the increased hindlimb splay relative to WT mice at the same exposure levels. Immunohistochemistry results showed severe degeneration of noradrenergic axons characterized by a dose-dependent decrease in the density of noradrenergic immunoreactive axons in NRF2-KO mice relative to WT mice. Moreover, body weight, whole brain weight and cerebellum weight were significantly reduced in NRF2-KO mice compared to the WT mice. The results suggest increased susceptibility to ACR-induced neurotoxicity in mice lacking the NRF2 gene. In conclusion, NRF2 is able to attenuate the effects of ACR-induced neurotoxicity in mice and thus remains a crucial target for the preventive modulation of neurotoxicity.

3086 The Effect of Polybrominated Diphenyl Ethers on Daf-18 Gene Expression in and NaCl Chemotaxis in *Caenorhabditis elegans*

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Autism Spectrum Disorder (ASD), affects 1 in 68 children in the United States, with no known cause. A combination of genetic and environmental factors may influence the increase incidence of the disease. A gene altered in some ASD patients is PTEN (Phosphatase and Tensin), a tumor suppressor responsible for cell cycle regulation that when dysregulated results in the overgrowth of neurons in ASD. One possible environmental factor linked to ASD is early exposure to polybrominated diphenyl ethers (PBDES). PBDE flame retardants used in foams, plastics, and some clothing, have been correlated with increased behavioral and cognitive deficits in children. The current study aims to elucidate a possible potentiation between PBDE exposure, PTEN expression, and development of ASD. The homologue for PTEN in *Caenorhabditis elegans* is DAF-18. Both the expression of Daf-18 and the effects of PBDEs on chemosensation, a behavior linked to Daf-18 expression were examined following exposure to BDE-47 in *C. elegans*. Real Time PCR was used to measure Daf-18 gene expression following chronic treatment of N2-wildtype worms with 20 µM BDE-47. For behavioral analysis, three test groups, N2 wildtype (control), N2 wildtype (PBDE treated) and the DAF-18 mutants (CB-1375 strain, positive control) were used in a NaCl chemotaxis assay. For both studies gravid adults were synchronized and allowed to hatch to the L1 stage before treatment. After treatment worms were fed and permitted to develop to the L4 stage. L4 stage worms were collected for RNA isolation and behavioral analysis. Multiple primers for Daf-18 were tested each showing upregulation of Daf-18 expression in the treated group compared to control. The chemotaxis was index calculated to determine the association of NaCl with food. At 60 min the chemotaxis index was negative for the PBDE treated worms indicating PBDEs may disrupt chemosensation. Results of the experiment showed that PBDEs can disrupt Daf-18 expression and alter behavior linked to this gene suggesting a possible potentiation of PTEN in ASD development.

3087 Assessing Possible Mechanisms of Neurotoxicity for Food Dyes Using ToxCast

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There is evidence that some chemicals used as food colorings may be harmful to children's health. In particular, emerging child health concerns pertain to the potential impacts on neurobehavioral and possibly other neurological endpoints. Data about health effects of food additives on infants and children are limited; however, in general, infants and children are more vulnerable to chemical exposures. This work focuses on using *in vitro* high-throughput screening (HTS) approaches to assess potential chemical effects of the food dyes across multiple cellular pathways. The study uses the HTS data from the US EPA's ToxCast™ database in an attempt to better understand the potential hazards of the nine food dyes that are currently "batch-certified" for use in the US. We evaluated the bioactivity of these dyes in two ways. One was to identify ToxCast assays targeting genes associated with neurologic activity. Genes include those for acetylcholinesterase, and receptors for glutamate, opiates, dopamine, and serotonin. The food dyes would then be screened for their activity in this ToxCast assay subset. Another approach consisted of profiling the bioactivity of pesticides known to be associated with developmental neurotoxicity and assessing whether there was an overlap between the bioactivity of these chemicals with those of the food dyes. Using information from these approaches, the nine food dyes are ranked by their bioactivity using the Toxicological Prioritization Index (ToxPi). ToxPi ranking was based on a sum of chemical-assay AC50 and inactive values. From our initial results, the ToxCast data support certain literature findings such as the interaction of Red 40 with cholinergic and dopaminergic receptors, as well as the interactions of Yellow 5 and 6 with serotonin receptors. Overall, yellow and red food dyes appeared to have the majority of assay activity. ToxCast results did not support reported Red 40 interactions with glutamate receptors, or reported Blue 1 and 2 interactions with cholinergic receptors. There was no activity for food dyes in assays targeting acetylcholinesterase, or GABA or glutamate receptors. Such findings highlight the ongoing challenges in the application of current *in vitro* data to reliably identify neurotoxic chemicals. Nonetheless, this initial assessment demonstrates the use of HTS to explore modes of action in connection to downstream toxicological endpoints in the absence of other data.

3088 Sub-chronic Exposure to Aircraft Engine Oils Decreases Acetylcholinesterase and Butyrylcholinesterase Activities in Brain Tissues, Blood, and Plasma for Sprague Dawley Rats

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Organophosphates are known to inhibit cholinesterase enzymes in animals and humans. However, little is known about the toxicity that may be associated with a sub-chronic exposure to organophosphate-based aircraft engine oils. This study was conducted to determine if grade 3 (G3) and 4 (G4) aircraft engine oils in their new states (G3-N and G4-N) and their used versions (G3-U and G4-U) have the potential to impact cholinesterase enzyme activities via dermal application. Male and female (n=50/gender) Sprague Dawley Rats (8-9 weeks old) were dermally exposed to water (control), new and used versions of G3 and G4 oils (10 males and 10 females/treatment) for a 21 day period to determine the oil's sub-chronic impact on cholinesterase enzymes in brain tissues, blood and plasma. Fifty recovery rats (n=25/gender; 5 rats/treatment) also received similar treatments and were kept for 14 days post-exposure to screen for reversibility, persistence, or delayed alteration in activities of these enzymes. A volume of 300 µL of oil was applied in the pad of the Hill Top Chamber System©. The chamber was attached to a fur-free test site located on the back of the rat for 6 h/day, 5 consecutive days/week for 21 days (15 total exposures). In brain tissues, both versions of G3 and G4 oils significantly ($p<0.05$) decreased (32% to 41%) the AChE activity for females. Only G3-N significantly lowered (33%) the AChE activity for males. G4-N did not cause a significant decrease in AChE. All these effects resolved by end of the recovery period. In whole blood samples, G3-U significantly decreased AChE by 29% for females at the end of exposure period and this effect persisted during the recovery period (32% decrease). Blood AChE activity for males was not affected. Butyrylcholinesterase (BChE) in plasma for males exposed to G4-N decreased by 29%, but resolved during the recovery period. In summary, AChE activities in rats were more altered than those for BChE and females were more sensitive to oil exposure than males. This study highlights potential health risks to which aircraft maintenance workers may be exposed if precautions are not taken to minimize exposure to these oils. *(In compliance with DODI 3216.01.)*

3089 Propofol Self-Administration Under a Progressive-Ratio Schedule in Rats and Rhesus Monkeys

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A progressive-ratio schedule is a procedure used to assess the strength of reinforcing effects in self-administration studies. In non-clinical research, propofol functions as a positive reinforcer in self-administration studies under fixed-ratio schedules in rats and rhesus monkeys. While clinical misuse and abuse of propofol has been reported worldwide, and some of these instances have resulted in death, non-clinical results using propofol under the progressive-ratio schedule have not yet been reported. Therefore, in this study, we compared the strength of reinforcing effects among cocaine, ketamine and propofol under a progressive-ratio schedule in rats and rhesus monkeys. In rats (n=4), the mean breaking point of cocaine at 1 mg/kg/infusion was 88.3 and was comparable with that of ketamine at 3 mg/kg/infusion. On the other hand, the mean breaking point of propofol at 3 mg/kg/infusion was just 8.3% that of cocaine. In rhesus monkeys (n=2), the mean breaking point of cocaine at 0.1 mg/kg/infusion was 975 and the mean breaking point of ketamine and propofol were 7.5 and 29.7% of cocaine, respectively. As described above, the strength of reinforcing effects were "cocaine = ketamine > propofol" in rats and "cocaine > propofol > ketamine" in rhesus monkeys. Therefore, when choosing animal species, it is important to note that differences among species may exist when comparing in the strength of reinforcing effects.

3090 Development of a High-Content Human Co-culture Model to Predict Chemotherapy Induced-Peripheral Neuropathy

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Antibody-drug conjugates (ADCs) are intended for targeted delivery of highly potent payloads to cancer cells. Tubulin-disrupting drugs are frequently used as ADC payloads and have been associated with a side effect of peripheral neuropathy (PN). Current nonclinical models of PN are practically challenging with uncertain translatability. Optimization of high-content (HC) systems offers the possibility of sensitive, cost-effective, and translatable tools for predicting potential PN in the clinic. ADCs may cause PN by a variety of mechanisms, including a bystander effect (BE) where cell permeable payloads may be released in a non-targeted way into adjacent tissue. Developing models that incorporate the BE requires the presence of support cells in the test system. We have characterized a novel, human cell-based, co-culture system comprised of induced pluripotent stem cells-derived sensory neurons (hSNs) and primary human Schwann cells (hSCs). A comparison of range of densities and ratios revealed 4:1 (hSNs:hSCs) as optimum for imaging and evaluation of neurite length and total cell number. We further characterized this system by evaluating dose responses for two known clinical neurotoxicants, paclitaxel and oxaliplatin. Analysis showed that the co-cultures were less sensitive than monocultures across all parameters tested, suggesting that support cells improve the viability of hSNs. Next, we exposed co-cultures and hSN monocultures to non-targeted ADCs conjugated with either monomethyl auristatin E (high BE) or monomethyl auristatin F (low BE). Differentiation between the neurite length in these systems, and an apparent increase in toxicity of MMAE non-targeted ADC in the presence of hSCs, points to BE as being a potential mechanism of action. These experiments demonstrated that a high-content, human cell-based, co-culture system could be an effective tool for assessing the PN potential of new therapeutics.

3090a Regulation of Synaptic Plasticity-Related Gene by Nicotine on Human Neuroblastoma SH-SY5Y Cells

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The nicotine addiction process shares many commonalities with the synaptic plasticity mechanisms normally involved in learning and memory. In this study, the changes of 84 synaptic plasticity-related genes in transcription level were investigated by treating SH-SY5Y cells with 2 different nicotine concentrations (6μM and 60μM) for 2 exposure time durations (12 hours or 24 hours). For 12 hours exposure, only 2 genes (CNR1, GRM5) were up-regulated after exposure to 6μM nicotine (the fold regulation is 2.28, 2.01), while 6 genes(CNR1, GRIA2, GRIN2A, GRM1, GRM8, NOS1) were up-regulated (the fold regulation is 2.55, 2.27, 2.81, 2.06, 3.35, 2.06) when the concentration of nicotine was 60μM. On the contrast, 6 genes (ARC, EGR1, EGR3, EGR4, GRM1, TNF) were down-regulated (the fold regulation is –2.23,–2.35,–2.35,–2.66,–2.17,–2.46) and only 1 gene(GRIN2B) is up-regulated (the fold regulation is 2.69) when the cells were exposed to 6μM nicotine for 24 hours, while 6 genes (GRIN1, GRIN2A, GRIN2B, GRM8, NGFR, NPTX2) were up-regulated (the fold regulation is 2.25, 2.66, 3.89, 7.73, 2.33, 2.43) and 1 gene (ARC) was down-regulated (the fold regulation is –2.22) after exposure to 60μM nicotine for 24h. These significant changed genes are associated with the formation of synaptic connections and the transmission of neural signals, suggesting nicotine plays an important role in the remodeling of synaptic structure and function in human neurons.

3091 Baseline Cardiac Phenotypes Vary across a Diverse Population: Implications for Cardiotoxicity Risk Assessment

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Cardiotoxicity is often the most sensitive parameter for determining threshold exposure levels of toxicants, but relatively few environmental chemicals have been tested successfully for cardiotoxicity. This is due, in part, to incomplete, although ongoing, development of models of cardiotoxicity, both *in vitro* and *in vivo*. Simplistic *in vitro* models lack receptor diversity, while *in vivo* models usually make use of a homogenous population. Of those chemicals tested, translating findings from current models to human risk assessment is challenging due to the lack of genetic diversity represented in these models, as well as limited epidemiological data to validate translation to humans. Animal models have been the gold standard for risk assessment, and with the use of individuals from a genetically diverse mouse reference population, the Collaborative Cross (CC), we can model the genetic diversity present in the human population. In 31 strains of mice, electrocardiogram measurements indicate a high strain-dependent variability ranging from 300 BPM to 800 BPM as well as significant differences in QT interval duration. Comparing cardiac high frequency ultrasounds acquired in both conscious and unconscious mice, strain-dependent effects of isoflurane on cardiac function were observed. Results demonstrate inter- and intrastrain variability in unexposed mice. While the goal is to create a powerful diverse testing panel whose baseline phenotypes are characterized before and after exposure to several chemicals, these baseline measurements are crucial for identifying the ideal model for testing toxicity, ultimately allowing more informative risk assessments.

3092 Triptolide Induces Cardiotoxicity by p53 Activation in Cardiomyocytes

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Triptolide (TP), a principal active ingredients extracted from Chinese herb *Tripterygium wilfordii* Hook. F. (TWHF), has attracted wide attention of its therapeutic effects on inflammation and autoimmune diseases. Severe cardiomyocyte toxicity is the main factor hindering its clinical application. Our studies indicated that p53 was indispensable in TP-induced cardiotoxicity. We investigated the role of p53 in TP-induced cardiotoxicity in H9c2 cell lines, primary neonatal rat cardiomyocytes, and $p53^{-/-}$ mice. p53 protein level was elevated by TP *in vitro* and *in vivo*. p53 deficiency effectively prevented heart histology injury, reversed myocardial energy metabolism and decreased serum cardiac troponin I (cTn-I). p53, as a transcription factor, regulates the transcription of its target genes involved in apoptosis, such as Bcl2 family members. On the other hand, p53 activation inhibites IKKβ-NF-κB pathway that regulates glucose transporter (GLUT) expression. Based on these evidences, we investigated the mechanisms of p53 mediated cardiotoxicity from two sides, motochondrial-dependent apoptosis and disturbed glucose metabolism. Immunoblotting and immunofluorescence identified that TP-induced toxicity was dependent on p53 nuclear translocation and transactivation of Bcl2 family genes leading to mitochondrial outer membrane permeabilization. p53 antagonist PFTα counteracted apoptotic gene transcription and improved mitochondrial membrane integrity. Bax inhibitor peptide (BIP) V5 ameliorated TP-induced apoptosis through suppressing membrane depolarization and ROS accumulation. Additionally, depressed glucose consumption and ATP production by TP also contributed to the cardiac damage. ATP improved TP-induced injury. Mechanically, TP suppressed glucose uptake by restriction of IKKβ-NF-κB activation, GLUT1 and GLUT4 expression which was abolished by PFTα treatment. Consistently, in acute heart injury models, *p53* deficiency upregulated IKKβ-NF-κB activation and GLUT1 and GLUT4 protein levels. The present findings indicated that p53 mediated TP-exerted cardiotoxicity by Bax-induced apoptosis and disturbing glucose metabolism through inhibition of GLUT1 and GLUT4 expression.

3093 Cardiovascular Toxicity Assessment of Tris (2-Chloroethyl) Phosphate (TCEP) in *Ex Ovo* Chicken Embryos

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Tris (2-chloroethyl) phosphate (TCEP) is one of pervasive organophosphate flame retardants (OPFRs) that have been used for textiles, industrial materials and furnitures. Some of avian species are top predators in the ecosystem and are exposed to high concentrations of environmental contaminants through the food web. TCEP has been detected from tissues and eggs of wild birds. However, there are fewer studies regarding the effects of TCEP on avian embryos. In *in ovo* exposure test of avian embryonic development, *in situ* observation has been difficult because avian embryos develop within untransparent eggshell. A novel shell-less incubation system for chick embryos that enables to visualize *in situ* the development of chicken embryos has been developed in 2014. Here, we assessed the developmental toxicity of TCEP exposure on chicken embryos in the shell-less incubation system. Fertilized chicken (*Gallus gallus domesticus*) eggs were treated with 50, 250 and 500 nmol/g egg of TCEP (TCEP-L, -M and -H, respectively) or DMSO (control) at the beginning of incubation. Survival rates started to be greatly reduced from 3rd incubation day and were significantly decreased until 9th day in TCEP-M and TCEP-H groups. The heart rate was significantly decreased on 5th day in TCEP-M and on 5th and 6th days in TCEP-H. The length and number of extra-embryonic blood vessels were significantly decreased on 4th day in TCEP-M and TCEP-H embryos. Digital gray image values of the extra-embryonic artery, an indicator of the number of red blood cells, were significantly decreased on 4th and 5th days in TCEP-M and on 4-7th days in TCEP-H. The cardiac transcriptome analysis and the following transcription factor enrichment analysis intimated that activation of NFKB1, SP1, SP3, GLI1, CTNNB1 and SMAD4 by TCEP exposure were critical molecular initiating events. Protein-protein interaction network analysis showed the dysregulation of genes related to muscle filament sliding and Ca^{2+} transport. In KEGG pathways, expression levels of ryanodine receptor 2 (RYR2) and, myosin genes were changed, suggesting altered cardiac muscle contraction. These results indicate that TCEP exposure to chicken embryos may induce decreases in heart rate and survival rate by altered expression of genes related to myocardial contraction.

3094 Effects of Small Molecule Protein Kinase Inhibitors on Isolated Rat Heart Mitochondria

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As of October 2019, the FDA has approved 53 small molecule kinase inhibitors (KIs), with 30 of them (57%) having warnings for cardiotoxicity in the labeling, 3 being boxed warnings. To better understand the mechanisms of KI cardiotoxicity, the mitochondrial liabilities of KIs were examined in isolated rat heart mitochondria. Freshly prepared mitochondria were treated with KIs at concentrations normalized to the peak blood concentrations (Cmax) in humans, and then oxygen consumption, inner membrane potential, swelling, cytochrome c release and reactive oxygen species were measured. In general, it was found that KIs, including ceritinib, lorlatinib and encorafenib, caused either significant inhibition of state 3 respiration or remarkable increases in state 4 respiration, indicating these drugs are either respiratory chain inhibitors or uncouplers. For the three KIs with boxed warnings for cardiotoxicity, only vandetanib remarkably inhibited oxygen consumption, and the other two drugs, nilotinib and ponatinib, showed no effects, suggesting the involvement of alternative mechanisms for their cardiotoxicity. A notable finding is that regorafenib caused almost complete uncoupling at concentrations 2-fold Cmax, and its two pharmacologically active metabolites, M2 and M5, whose blood levels are the same as regorafenib, were remarkably less toxic to the mitochondria, indicating that regorafenib cardiotoxicity is likely mainly associated with the parent drug but not the metabolites. These data suggest that off-target effects on mitochondrial functions may contribute to KI induced cardiotoxicity.

3095 Cardiac Toxicity Comparison of Roundup and Glyphosate on Human-Induced Pluripotent Stem Cells Derived Cardiomyocytes

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Pesticides are widely widespread in agriculture but their use is suspected of hazardous health effects. Evaluate the safety and potential health risks from exposure to pesticides is a major challenge. Roundup® is an herbicide formulation based on an active ingredient, glyphosate, and adjuvants. Roundup® and glyphosate were compared using human cell based model to evaluate their impacts on cardiovascular function after exposition. Several concentrations of Roundup® and glyphosate were recorded on iCell² cardiomyocytes (derived from Human induced Pluripotent Stem Cells, Fujifilm) using the xCELLigence RTCA cardio ECR platform. Cardiac contractility (impedance) and electrophysiology (MEA) were simultaneously monitored during acute and chronic expositions (up to 24h). No modification of cardiac contractility nor electrical activity or cell viability have been observed in the presence of glyphosate even at the highest concentration of 100µM. At the same concentration of Roundup®, iCell² cardiomyocytes stopped beating and cell index was rapidly and drastically affected (-55% after 24h), corresponding to partial cardiomyocytes death. The absence of effects of glyphosate on cardiac function indicates that the cardiotoxicity observed with Roundup® could be attributed to Roundup® adjuvants. This predictive and sensitive assay can be very useful for cardiotoxicity assessment of pesticides using a human cell based model. This work also demonstrates that the evaluation of pesticides' adjuvants is also necessary to better address the safety of pesticides which can also be enlarged to a broader class of molecules.

3096 Arsenic Is a Unique Modulator of Macrophage Polarization toward Pro-Atherogenic Phenotypes

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Arsenic toxicity is a global health concern and is correlated with adverse cardiovascular outcomes, including atherosclerosis. Atherosclerotic plaque formation is a complex process with macrophages being major players in both its initiation and progression. Macrophages accumulate lipids and modulate the microenvironment through secreting cytokines and recruiting other immune cells to the site. There is phenotypic heterogeneity of macrophages residing in plaques, the most prevalent of which are broadly characterized as M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes in response to microenvironmental cues. Here, we hypothesized that arsenic exerts proatherogenic effects by skewing the relative abundance of pro- and anti-inflammatory macrophages towards pro-inflammatory M1 macrophages. To test this hypothesis, we cultured bone marrow derived macrophages from C57BL/6 mice *in vitro* and differentiated them into M1 (IFNγ) or M2 (IL-4) phenotypes in the presence or absence of 50 ppb arsenic over 48 hours. Gene expression was assessed by RNA sequencing. The principle component analysis showed that unpolarized (M0), M1, and M2 macrophages could easily be distinguished. Surprisingly, arsenic did not result in a separate population of "arsenic-exposed macrophages", but rather altered different gene expression within each subtype. Within M2 macrophages, many chemokines were altered by arsenic. In particular, CCL17 and CCL22 mRNA and secreted CCL17 and CCL22 protein levels were decreased in the presence of arsenic. These anti-inflammatory chemokines recruit Tregs leading to the plaque resolution. We will correlate arsenic exposures with decreased CCL17/22 to decreased Tregs and increased plaque size *in vivo*.

3097 Transient and Sustained Antioxidant Protection in Progeny following Gestational Engineered Nanomaterial Inhalation Exposure

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Increased utilization of engineered nanomaterials (ENM) necessitates thorough investigation into the systemic toxicological events associated with inhaled exposure. Of particular concern are vulnerable populations, such as the developing fetus, which may experience more detrimental alterations following maternal exposure. This study aimed to determine whether targeting antioxidant defense to the mitochondrion via overexpression of mitochondria phospholipid hydroperoxide glutathione peroxidase (mPHGPx) could diminish cardiac dysfunction in fetal and adult progeny following maternal exposure to nano-TiO_2 during gestation. To gain insight into the importance of maternal vs progeny regulated mitochondrial antioxidant protection during development, two strategies were employed. Wild-type (WT) and transgenic (mPHGPx) female mice were bred with transgenic and WT males, respectively. Pregnant dams were exposed to sham air or nano-TiO_2 (primary particle size = 21 nm, mass concentration = 12 mg/m^3) for 6 hrs/day over 6 days. Echocardiography performed at the fetal stage (GD 14) revealed that maternal antioxidant protection significantly enhanced cardiac output by 86% and stroke volume by 43% compared to pups of nano-TiO_2-exposed

WT dams. At the adult stage (11 wks), transgenic offspring of WT dams presented with significantly higher ejection fraction (8%) and fractional shortening (29%) compared to WT offspring of nano-TiO_2-exposed transgenic dams. Therefore maternal antioxidant protection, in the absence of the transgene in the offspring, may not be sufficient for complete protection into adulthood. Mitochondrial respiration revealed a >3 fold higher respiratory control ratio in transgenic offspring of transgenic dams compared to transgenic offspring of WT dams. N^6-methyladenosine (M^6A) abundance, which can be induced by elevated ROS, was significantly decreased in the WT offspring of transgenic (0.046 ± 0.007) and WT dams (0.050 ± 0.009) when compared to WT sham-exposed offspring (0.091 ± 0.008). These data present the potential of antioxidant defense during fetal development and adulthood for ameliorating cardiac dysfunction following gestational ENM exposure, yet highlight the pivotal role of antioxidant delivery context.

PS 3098 Sex-Biased Differences in Circulating microRNAs in Doxorubicin-Treated Mice

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Despite a severe side effect of cardiotoxicity, doxorubicin (DOX) remains one of the most widely used anticancer drugs. There is evidence that males are more susceptible to DOX-induced cardiotoxicity than females. While a majority of animal studies have focused on heart tissue, information on sex-biased circulating microRNAs (miRNAs) is lacking. Circulating miRNAs are widely considered as potential markers of many cardiac diseases or toxicities because of their role as key regulators of gene expression and important biological processes. We have previously shown that a cumulative dose of 24 mg/kg DOX caused cardiac pathology and significantly altered miRNA expression in male B6C3F1 mouse heart. Therefore, male and female B6C3F1 mice (n=8) were treated with a weekly dose of 3 mg/kg DOX or saline (SAL) via tail vein for 8 weeks (resulting in 24 mg/kg cumulative dose). At necropsy one week after the last dose, plasma samples were collected to evaluate sex difference in the level of circulating miRNAs in response to DOX-treatment leading to cardiotoxicity. MiRNA sequencing was performed on Illumina NextSeq system using Edgeseq Whole Transcriptome Assay for 273 mouse miRNAs. With a cutoff of FDR<0.1, 143 plasma miRNAs were significantly altered (DOX vs. SAL) in males, while only 1 miRNA was significantly altered in female mice. Six miRNAs (miR-130a, miR-19b, miR-21, miR-221, miR-23b, miR-34a) had at least 1.8-fold higher transcript level (DOX vs. SAL) in male plasma and these were also significantly upregulated (FDR<0.1) in the hearts of male mice treated with the same dose of DOX. Enrichment analysis of 143 significantly altered miRNAs in males and their mRNA targets revealed involvement of many plasma miRNAs (such as miR-21, miR-19b, miR-221) in cardiac hypertrophy, fibrosis, necrosis and apoptosis. Supporting these findings, a greater susceptibility of male hearts to DOX compared to females was indicated by a 3.6-fold increase in plasma cardiac troponin T in male mice compared to females and cytoplasmic vacuolization only in male hearts. These circulating miRNAs may shed light on the mechanisms of differential sex-biased susceptibility to DOX toxicity and may have potential in predicting early-onset of DOX cardiotoxicity.

PS 3099 Prediction of Drug-Induced Cardiotoxicity and Liver Toxicity Using Chemical Structure and *In Vitro* Assay Data

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Drug-induced cardiotoxicity and liver toxicity (DILI) are major adverse effects encountered by many clinically important drugs especially antineoplastic agents. To provide an alternative to *in vivo* toxicity testing, the US Tox21 program has screened a collection of ~10K compounds, including drugs in clinical use, against ~70 cell-based assays in a quantitative high-throughput screening (qHTS) format. In this study, we evaluated the potential of Tox21 assay data in comparison with chemical structure information in building optimal prediction models for human *in vivo* cardiotoxicity and DILI. Models were built with a number of machine learning algorithms (e.g., random forest, SVM, MLP) and model performance was evaluated by area under the receiver operating characteristic (ROC) curve (AUC) and Matthews Correlation Coefficient (MCC). Chemical structure based models showed moderate predictive power for cardiotoxicity (AUC = 0.69±0.04) and better predictive performance for DILI (AUC = 0.77±0.05). Tox21 assay data alone only showed better than random performance with AUCs around 0.6. Combining assay data and structure information significantly improved the performance of the cardiotoxicity prediction model (AUC = 0.79±0.06) but did not have a positive impact on DILI prediction. The suboptimal predictive performance of the assay data is likely due to insufficient coverage of an adequately-predictive number of toxicity mechanisms. Tox21 is currently expanding the coverage of biological response space with additional assays that probe toxicologically-important targets and under-represented pathways that may improve the prediction of *in vivo* toxicity such as DILI and cardiotoxicity.

PS 3100 Protective Effect and Mechanism of Metallothionein on Heart Damage Induced by High-Fat Diet and Therapeutic Dose of Arsenic Trioxide

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Arsenic trioxide is a common clinical anti-tumor drug. However, the cardiotoxicity caused by its application seriously hindered the strategy of treatment. Unfortunately, patients with tumors also commonly suffer from heart damage induced by a high-fat diet or abnormal lipid metabolism, which greatly limits the choice of arsenic trioxide as an anti-tumor treatment. Metallothionein is an effective endogenous antioxidant, which can effectively protect cardiac myocytes from oxidative stress injury. Therefore, the purpose of this study was to prove whether metallothionein can protect cardiac myocytes and inhibit the occurrence of cardiac toxicity under the double attack of high-fat diet and treatment dose of arsenic trioxide. MT-TG transgenic and WT mice were exposed to a high-fat diet and a normal diet. Subsequently, arsenic trioxide (5mg/kg/day) was injected for one month. Metallothionein has a protective effect on myocardial oxidative stress injury, which may be achieved by directly inhibiting oxygen free radicals. In addition, metallothionein protects myocardial cell apoptosis by inhibiting the P53 signaling pathway and inhibiting myocardial cell apoptosis. Finally, metallothionein can inhibit the recruitment of immune cells by inhibiting the activation of the NF-kB inflammatory signaling pathway and inhibiting its downstream inflammatory cytokines, adhesion molecules and chemokines, thus achieving the protective effect of myocardial inflammatory response and fibrosis. 1.High-fat diets increases the sensitivity of ATO leading to heart damage. 2.MT inhibits cardiomyocyte fibrosis, apoptosis, inflammatory response, and oxidative stress injury induced by high-fat diets and therapeutic doses of ATO.3.The protective mechanism of MT on the heart may be achieved by directly reducing oxygen free radical production.

PS 3101 Effects of Polysorbate 80 as a Vehicle: Species Comparison in Beagle Dogs, Cynomolgus Monkeys, and Göttingen Minipigs

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Polysorbate 80 is a nonionic surfactant and emulsifier commonly used as a vehicle in non-clinical formulations. Polysorbate 80 has been associated with IgE independent anaphylactoid reactions in various species including humans, with dogs recognized as the most susceptible species. Polysorbate 80 at 3 mg/kg (0.15%, 2 mL/kg, 15 min IV infusion) was administered to Beagle dogs, Göttingen minipigs and cynomolgus monkeys. Dogs were also treated with Polysorbate 80 at 3 mg/kg as a 15 min IV infusion with diphenhydramine (1 mg/kg, SC) pre-treatment. Additionally, Polysorbate 80 at 10 mg/kg was administered orally to Beagle dogs. All animals were monitored for clinical signs or cardiovascular changes by telemetry. No clinical signs, histamine level changes or cardiovascular effects were observed in cynomolgus monkeys or Göttingen minipigs. In dogs, a 3 mg/kg 15 min IV infusion was associated with a body temperature decline reaching a -2 Celsius nadir at 45 min post-dose and recovery to baseline comparable values by 105 min post-dose. Hypotensive effects were transient but chronotropic changes were sustained. Parallel to the cardiovascular changes, mean plasma histamine levels were maximal at 20 min post-dose at 3 mg/kg and diphenhydramine was associated with a 3-fold reduction in histamine release and a significant attenuation of cardiovascular effects. Oral administration of Polysorbate 80 at 10 mg/kg was associated with facial edema in 33% of dogs but without any cardiovascular changes. Polysorbate 80, at 3 mg/kg as 15 min IV infusion was not associated with any signs of anaphylactoid reaction in cynomolgus monkeys or Göttingen minipigs. In dogs, skin edema was observed prior to cardiovascular changes and diphenhydramine pre-treatment significantly attenuated cutaneous and cardiovascular changes. Overall, these results confirm the high susceptibility of Beagle dogs to Polysorbate 80 mediated anaphylactoid reactions with differences between dosing routes.

3102 Weighted Gene Coregulation Network Analysis (WGCNA) to Decipher Temporal Dynamics in Cardiotoxicity

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Cardiotoxicity can be elicited through a variety of mechanisms, which lead to complex, temporally regulated compensatory processes at the molecular level. The sequence of molecular processes has evolutionary roots that, at a regulatory level, are analogous to highly complex agent-based models with self-organized behavior. One approach to delineating the evolved, self-organizational framework is to characterize the coordinated expression genes across a diverse set of toxicological perturbations using WGCNA. Here we describe a cartographic exercise that uses WGCNA of the DrugMatrix heart gene expression data (1-5 days in duration, 88 test articles) from rat to reveal a map of gene-level co-expression relationships that are rooted in compensatory/adaptive processes associated with cardiotoxic stress. In total, the map reveals sets of genes tightly linked to well document biological processes such as cell cycle and ribosomal biogenesis, but also identifies sets of genes that are reflective of changes in cellularity and processes central to the differentiate functions of cardiac cells. We use the co-expression map as a base framework to explore the temporal dynamics of cardiotoxicity of a variety of prototype agents such as anthracyclines, corticosteroids, and kinase inhibitors. The exercise reveals early agent specific behavior that evolves into a general compensatory process that is likely intrinsic to the reparative function of the heart and is conserved across most test articles. We believe this analysis serves as molecular level point of reference that can be used to understand the capabilities and limitations of *in vitro* systems for modeling cardiotoxicity.

3103 A Targeted Metabolomics-Based Assay Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Identifies Structural and Functional Cardiotoxicity Potential

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Implementing screening assays that identify functional and structural cardiotoxicity earlier in the drug development pipeline has the potential to improve safety and the cost and time required to bring new drugs to market. In this study, a metabolic biomarker-based assay was developed that predicts the cardiotoxicity potential of a drug based on changes in the metabolism and viability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Assay development and testing was conducted in two phases: (1) biomarker identification and (2) targeted assay development. In the first phase, metabolomic data from hiPSC-CM spent media following exposure to 66 drugs was used to identify biomarkers that identified both functional and structural cardiotoxicants. Four metabolites that represent different metabolic pathways (arachidonic acid, lactic acid, 2′-deoxycytidine, and thymidine) were identified as indicators of cardiotoxicity. In phase two, a targeted, exposure-based biomarker assay was developed that measured these metabolites and hiPSC-CM viability across an eight-point concentration curve. Metabolite-specific predictive thresholds for identifying the cardiotoxicity potential of a drug were established and optimized for balanced accuracy or sensitivity. When predictive thresholds were optimized for balanced accuracy, the assay predicted the cardiotoxicity potential of 81 drugs with 86% balanced accuracy, 83% sensitivity, and 90% specificity. Alternatively, optimizing the thresholds for sensitivity yields a balanced accuracy of 85%, 90% sensitivity, and 79% specificity. This new hiPSC-CM-based assay provides a paradigm that can identify structural and functional cardiotoxic drugs that could be used in conjunction with other endpoints to provide a more comprehensive evaluation of a drug's cardiotoxicity potential.

3104 The Effects of Inhaled Multiwalled Carbon Nanotubes on Systemic Blood Pressure and the Autonomic Nervous System in Spontaneously Hypertensive Rats

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It is well documented that the autonomic nervous system (ANS) plays a critical role in controlling cardiovascular functions including heart rate, cardiac contractility and blood pressure (BP). In previous studies, we reported that inhalation of multi-walled carbon nanotubes (MWCNTs) elevated systemic BP, which was associated with an altered activity in the ANS in normotensive rats. In addition, we also reported that the BP of spontaneously hypertensive (SH) rats was greatly increased in response to pulmonary MWCNT exposure compared to normotensive rats. The available evidence indicates that in essential hypertension, there is a deteriorated balance between sympathetic and parasympathetic activity. The present study investigated the effects of inhaled MWCNTs on the ANS and the role of peripheral neurons in regulation of cardiovascular function after exposure of SH rats to MWCNTs. SH Wistar rats were pre-implanted with a telemetry device and exposed by inhalation to MWCNTs at a concentration of 2 mg/m^3 for 5 h/day for three consecutive days. The real-time EKGs and systemic BP were recorded by a telemetry system at pre-exposure, during exposure, 1 day post-exposure and 7 days post-exposure. The activity of the ANS in response to MWCNT exposure was determined by heart rate variability (HRV) analysis. The non-selective transient receptor potential (TRP) channel blocker, ruthenium red (2.5 mg/kg), was injected intraperitoneally 1 h before MWCNT exposure to study the role of peripheral neurons in regulating cardiovascular function and activity of the ANS after pulmonary MWCNT exposure. Inhalation of MWCNTs elevated systemic BP and increased the variance of the root mean square of successive differences (RMSSD) between adjacent R-R intervals ($p < 0.01$) and the high frequency (HF) power ($p < 0.01$). Both RMSSD and HF are metrics corresponding to autonomic nerve influences on cardiovascular function. Pretreatment with ruthenium red prevented those increases. Our study indicates that pulmonary exposure to MWCNTs significantly altered the activity of the ANS and increased the BP via a peripheral neuron-regulated pathway in SH rats.

3105 Cerebrovascular Dysfunction and Microvessel Density Changes in Offspring of Rat Dams Exposed to Electronic Cigarette Aerosols

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Electronic cigarette (E-cig) usage has steadily been increasing and it is even being promoted as a safer option to traditional tobacco cigarettes; however, there is little evidence supporting this theory as it relates to the cerebrovasculature. We examined the effects of maternal E-cig exposure (Joyetech eGrip OLED using 5-sec puffs @17.5 W) on cerebrovascular function and microvessel density in offspring with maternal exposure to ambient air (control, n=6), E-cig with 18 mg/ml nicotine (E-cig18, n=6), and without nicotine (E-cig0, n=7). Exposure consisted of 60 puffs over 1-hour each day, 5 days/week, and resulted in an average daily TPM of ~120 mg/m^3. Maternal exposure was started on gestational day 2 and continued until pups were weaned. Pups themselves we never directly exposed. The middle cerebral arteries (MCA) were obtained from 3-month old pups, isolated and positioned in a pressurized myobath, and exposed to increasing concentrations of acetylcholine (Ach; 10 -9 M to 10 -4 M), serotonin (5-HT; 10 -9 M to 10 -4 M), and sodium nitroprusside (SNP; 10 -9 M to 10 -4 M), in the presence or absence of Tempol (a superoxide dismutase mimetic). Brains were also flash frozen, sectioned, and analyzed for microvessel density (MVD). The MCA dilation of offspring to Ach was impaired in both E-cig0 and E-cig18 by 63% and 62%, respectively, compared to controls (<0.05). Incubation with tempol reversed the cerebrovascular dysfunction seen in both E-cig groups, suggesting the superoxide pathway is involved in the impairment observed in offspring with maternal E-cig use. The MCA dilation to SNP and constriction to serotonin was similar between all groups. Preliminary data (n=2 per group) shows a 12% and 29% decrease in MVD in the cortex of E-cig0 pups and E-cig18 pups, respectively. These data suggest that E-cig usage during pregnancy impairs the cerebrovascular reactivity and induces rarefaction of cortical microvessels in offspring.

3106 Developing Patient-Centric *In Vitro* Cardiovascular Safety Models Applicable to Drug Discovery

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Translation of functional and structural toxicity findings from healthy to disease states is a major challenge to providing safe and efficacious drugs. Currently, a range of *in vitro* and *in vivo* model systems are utilized to risk assess cardiovascular safety. Largely, these approaches rely on cells derived from 1-2 healthy donors or healthy animals that do not recapitulate patient's co-morbidities, pathophysiology of disease, drug treatment history or genetic variability. Critical to cardiomyocyte function is calcium signalling for which genetic variability within key calcium handling genes in the population exists. We hypothesized that hiPSC-CMs containing SNPs or reduced expression levels of a critical calcium handling gene, *RYR2* would display differential pharmacological responses compared to isogenic controls. This model would

allow, elucidation of the impact of genetic variability in calcium handling on the manifestation of drug-induced cardiotoxicity. hiPSCs were transfected with Cas9-GFP and gRNA plasmids +/- ssODN, followed by single cell FACS sorting, expansion and verification of genotype by Amplicon/Sanger sequencing. The isogenic lines obtained (2 heterozygous knockouts (Het KO), 1 SNP1 and WT parental) contained 95-98% Oct4 and SSEA4 double positive cells as measured by FACS and immunofluorescence indicating a pluripotent state, had normal G-banding karyotype and could differentiate to beating hiPSC-CMs containing 70-90% of cTnT positive cells. In addition, the resulting hiPS-CMs expressed α-actinin, PLN and SERCA2. Furthermore, western blot analysis confirmed that Het KO *RYR2* lines had 2-fold reduction of RYR2 expression. Functional characterisation confirmed a stable beat rate and amplitude, in addition, WT and Het KO cells responded to electrical stimulation up to 2Hz. hiPSC-CMs were analysed for contractility changes in response to selected inotropes. For example, WT, Het KO or SNP1 cells were treated with the L-type calcium channel inhibitor, verapamil or vehicle control and beat rate IC_{50} values were determined. SNP1 cells displayed increased sensitivity compared to WT and Het KO cells. In conclusion, a novel patient-centric *in vitro* cardiovascular safety model was developed which displays differential cardiotoxic susceptibility response to drug treatment. This provides a proof of principle for patient-centric *in vitro* human models in cardiovascular safety.

3107 QA Interval Heart Rate Correction: Multispecies Comparison of an Indirect Ventricular Contractility Biomarker

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Ventricular contractility parameters are influenced by multiple factors (e.g. preload, afterload and heart rate). Recently, we described the value of using a multi-parameter correction on a key contractility parameter (i.e., dP/dt_{max}) for enhancing data interpretation in non-clinical drug safety assessments. To further refine cardiac contractility measurement strategies, we have assessed the potential utility of a heart-rate corrected QA interval (QAI) to that of dP/dt_{max} across three non-clinical animal species (rats, n = 13; dogs, n = 5; non-human primates, n = 13). The QAI is the time interval from the Q wave on the electrocardiogram (ECG) to the initiation of the upstroke in aortic pressure and represents an indirect measure of cardiac function, inversely proportional to direct cardiac contractility measures, and may act as a useful indirect index of cardiac contractility. As expected, higher heart rate values were associated with shorter QAI across all three species whereas dP/dt_{max} values were positively correlated to heart rate as previously reported. The correlation between the QAI and dP/dt_{max} evaluated by linear regression suggest that in non-human primates ($y = 32.24*x + 61.25$) and dogs ($y = 26.49*x + 18.18$) heart rate related changes in QAI are nearly independent of dP/dt_{max} whereas in rats a strong negative correlation exists between the two parameters ($y = -704.1*x + 5.113$). Upon application of a heart-rate corrected formula, both QAI and dP/dt_{max} displayed strong individual corrections reducing the slope of each respective trend line to near 0. Interestingly, corrected QAI values were noted to be more consistently corrected to values closer to 0 than dP/dt_{max} suggesting that heart-rate corrected QAI values may present a more consistent heart rate dependence. Taken together, heart rate corrected dP/dt_{max} and QAI can improve assay sensitivity to detect drug induced inotropic effects in order to assess drug-mediated effects on cardiac contractile function for use in non-clinical drug safety assessments. This observation maybe critical in the context of the high prevalence of chronotropic effects in drug safety testing.

3108 Developmental Exposure to Environmentally Related Concentrations of PCB126 and TPHP Exhibits Synergistic Effect on Vascular Growth of Zebrafish Embryos

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Persistent organic pollutant 3'3,4,4'5-pentachlorobiphenyl (PCB126) and organophosphorus flame retardant triphenyl phosphate (TPHP) are ubiquitous organic environmental pollutants. These two chemicals coexist in various environmental media and human samples and thus may have combined effects on human health. However, the vascular toxicity and related mechanism of co-exposure to PCB126 and TPHP remain unknown. High content imaging was performed on embryos at 72 hours post fertilization (hpf). A dose-response relationship was analyzed by benchmark dose (BMD) modeling. The results showed that the growths ofdorsal aorta (DA), subintestinal veins (SIV), and intersegmental vessels (ISV) were affected at doses ranging from 0-300 ug/L of PCB126 or 0-2400 ug/L of TPHP at 72 hpf, while no significant change was shown in common cardinal veins (CCV). DA was the most sensitive among the observed blood vessels to PCB126 or TPHP exposure. The BMD value of PCB126 and TPHP was 0.28 μg/L and 291.4 μg/L respectively. Compare to the exposure of chemical alone, co-exposure to both PCB126 and TPHP resulted in a more reduction of DA diameter and the gene expression of *VEGFA*, *VEGFR2*, as well as the DA diameter of BMD value for PCB126 and TPHP respectively. The results suggest that PCB126 and TPHP exert vascular toxicity and co-exposure led to synergistic effect in vascular development. The study provides new insights into the combined toxicity of exposure to polychlorinated biphenyls and organophosphorus flame retardants.

3109 Acute Effects of Electronic Cigarette Aerosol on Cerebral Perfusion in Mice

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Electronic cigarettes (E-cig) have become exceedingly popular with the campaigning notion of being more safe than tobacco cigarettes. However, it remains uncertain whether or not these products are less harmful than tobacco cigarettes. We analyzed the cerebral perfusion flux (CPF) using a Moor Full-Field Laser Perfusion Imager (FLPI)-2 in wild-type C57BL/6 mice by collecting one image every minute for up to 2 hours after exposure. Mice were anesthetized with Isoflurane (5% induction/ 2-3% maintenance). Five baseline (pre-exposure) images were taken prior to exposure to either, French Vanilla flavored E-liquid with 18 mg/ml nicotine (E-cig18, n=4) or without nicotine (E-cig0, n=3), Univ. of Kentucky-reference tobacco cigarette (3R4F, n=2), or ambient air (Sham, n=4). Exposures were for ~5 minutes (total of 10 puffs, with 1 puff every 30 secs). Separate Harvard Apparatus Dual Phase Control Respirator Pumps were used to administer the E-cig aerosol (from 3rd generation tank-style device set at 17.5 W) and cigarette smoke (from one 3R4F cigarette). Baseline images obtained prior to E-cig exposure were averaged and used for comparison to post-vaping values (repeated measures ANOVA). A rapid increase in whole-brain CPF was observed in E-cig0, E-cig18, and 3R4F immediately after vaping, showing peak responses of 13±9%, 12±1%, 9±7%, respectively (mean % change±SEM). CPF returned to baseline at 34 min, 16 min and 16 min in E-cig0, E-cig18, and 3R4F, respectively. There was no significant change in CPF in the Sham group (0 ± 2%) during the same time period as reported for the other exposure groups. The immediate increase in CPF was similar between E-cig (with or without nicotine) and tobacco cigarette, indicating that the cerebral vascular response to vaping is similar to smoking. These data suggest that the cerebral vasculature may not be able to differentiate between E-cig aerosol and cigarette smoke, and therefore is likely to lead to the same cerebral pathologies known to occur with smoking.

3110 Polyethylene Glycol (PEG)-Coated Liposomes Disrupt Endothelial Function via Alterations in Oxidative Stress

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Polyethylene glycol (PEG) liposomes are being investigated for improvement of drug delivery; however, the vascular toxicity associated with these modified liposomes is largely unknown. Previous research has shown that PEG coated liposomes are associated with vascular tissue up to 3 days post-exposure. Therefore, we hypothesize that this association will result in increased oxidative stress and ultimately impair microvascular function. Microvascular endothelial cells were treated with PEG and non-PEG coated liposomes (3, 50, and 100 ug/ml). Reactive oxygen species (ROS) generation was monitored via a CellROX kinetic assessment. Oxidative stress potential was assessed by analyzing the ratio of glutathione disulfide (GSSG) to glutathione (GSH) via HPLC. For *in vivo* assessments, Balb/C mice were injected with PEG coated or non-PEG coated liposomes (5mM) and mesenteric arterioles were harvested 3 days post exposure. Endothelium-dependent reactivity was assessed with acetylcholine (ACh, 10^{-9}-10^{-4} M) via pressure myography. After initial assessments, superoxide and hydrogen peroxide were scavenged in the presence of the superoxide dismutase mimetic 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL, 10^{-4} M) and catalase (50 U/mL). There was a significant increase in ROS following exposure to PEG-coated liposomes compared to the non-PEG liposomes and control (2.7 ± 0.1% fold-change vs. control 1.7 ± 0.1%). There was also an increase in oxidative stress potential in the endothelial cells exposed to PEG-coated liposomes as determined by an increase in the GSSG:GSH ratio (-229 ± 2 vs. control -118 ± 4). Finally, following only PEG-coated liposome delivery, endothelium-dependent dilation was significantly attenuated (-3.9 ± 24% vs. control 69 ± 4%), and this impairment was resolved following treatment with TEMPOL and catalase (41 ± 19% vs. control 69 ± 4%). Taken together, these results indicate that PEG-coated liposomes induce microvascular dysfunction and these impairments may be due to changes in oxidative stress and/or endothelial cell signaling. The understanding of

liposomes toxicity *in vitro* and *in vivo* is critical, since liposomes are being increasingly used as therapeutic delivery methods and adverse endothelial cell impacts may lead to adverse cardiovascular responses.

PS 3111 Critical Time Point of Gestational Nanomaterial Inhalation Exposure: Alteration of Angiotensin II Response within the Maternal Uterine Microcirculation

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Engineered nanomaterials are utilized in diverse applications yet cardiovascular ramifications of gestational exposure are poorly understood. The aim of this study was to assess microvascular and reproductive consequences of gestational inhalation exposure to nano-titanium dioxide (nano-TiO_2) during distinct trimesters. Our hypothesis was that time-points of gestational exposure cause varying degrees of microvascular dysfunction. Uterine microvascular angiotensin II (ang II) reactivity was assessed, as it is a critical vasoactive hormone involved in the microvascular adaptations to pregnancy. Ang II signaling occurs via the type 1 and type 2 receptors (AT_1R: constriction, AT_2R: dilation). Receptor balance during gestation is critical for maternal and fetal health. Sprague Dawley rats were randomly assigned to 6 nano-TiO_2 exposure groups (early gestational days (GD) 2-6, mid GD 8-12, late GD 15-19, or control groups). Evonik-P25 (primary diameter = 21 nm) was the bulk nano-TiO_2 material utilized. Whole-body exposure (concentration = 12±0.5 mg/m^3) was performed for 6 hours/d for 3 days (cumulative calculated lung burden = 263±16 µg). Rats were euthanized on GD 20. Pup characteristics were assessed and pressure myography was performed on isolated uterine radial arteries. Average dry pup weight of early exposure group was 0.40±0.02 g, 0.50±0.02 g for the mid exposure, and 0.63±0.02 g for the late exposure. All exposure group dry pup weights were significantly different, suggesting the earlier exposures reduce pup weight. Placental efficiency was significantly different among exposure groups (early exposure 3.3±0.2, mid 3.8±0.2, late 7.5±0.4). Radial artery ang II ($1x10^{-13}$ - $^{-4}$ M) reactivity was assessed via pressure myography. Reactivity was significantly different among exposure groups (average difference of max response: 15±5% early vs late, 8±3% early vs mid, 5±2% mid vs late). Experiments are ongoing to assess additional microvascular reactivity data. Additionally, immunohistochemistry is being performed on placental tissues to determine AT_1R and AT_2R distributions, which may account for the altered ang II sensitivity and may help pinpoint the mechanism of dysfunction. *Support: ES015022 (TRN) U54GM104942 (SH).*

PS 3112 High-Fructose Diet Increases Arrhythmia and Alters Ventricular and Hemodynamic Function One Day after a Single Wood Smoke Exposure in Wistar-Kyoto Rats

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It is well established that exposure to air pollution, including smoke from extreme events like wildfires, can worsen cardiovascular function and lead to adverse health responses. On the other hand, it is still not clear how certain diets affect these responses or the risk of developing long-term disease. This study examined the impact of a high-fructose (HF) diet on cardiovascular function and subsequent responsiveness to wood smoke inhalation in rats. We hypothesized that HF diet would lead to baseline cardiovascular mechanical changes and increased electrical dysfunction. Eight-week old Wistar-Kyoto rats were placed on either a normal (ND) or HF diet for seven weeks and then exposed to either filtered air (FA) or 0.5 mg/m^3 of flaming eucalyptus wood smoke (WS) for one hour. One day after exposure, rats were anesthetized, implanted with an intraventricular Millar probe and then challenged with dobutamine to determine cardiac and arterial function under stress conditions. There was no difference in the left ventricular (LVP) and aortic pressures between ND and HF rats, although exposure to WS caused them to increase significantly in both groups. HF rats had significantly higher heart rates (HR), stroke energetics, and stroke volume than ND. Although Tau or time of active relaxation was the same for FA-exposed ND and HF rats, WS caused it to increase more in the latter. On the other hand, WS only increased arterial stiffness in ND rats. During dobutamine challenge, WS increased the HR response in both ND and HF rats compared to controls, but caused a greater increase in LVP in the latter. HF rats had a significantly greater number of cardiac arrhythmias than ND and exposure to WS showed a trend of increase in these counts for both groups. Although these alterations do not necessarily represent the development of disease, they indicate a shift from the normal, which becomes more evident after exposure to WS. A HF diet may predispose the cardiovascular system to greater dysfunction upon a single exposure to wood smoke and has implications for public health impacts during biomass smoke events. *This abstract does not reflect US EPA policy.*

PS 3113 Proarrhythmic Assessment of Drugs Using Chronically Paced Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are commonly used to evaluate cardiac function *in vitro*. In a recently published report (Blinova K et al 2018), ten sites participated in Phase II Comprehensive *in vitro* Proarrhthmia Assay (CiPA) initiative and tested 19 arrhythmogenic and 9 non-arrhythmogenic compounds in 5 electrophysiology platforms and 2 hiPSC-CMs cell lines. Using the results, the authors developed regression models to assess risk probability. Albeit imperfect, these models correctly categorized 22 of the 28 compounds across the 10 participated sites. Depending on the cut-off value, these 9 drugs were miscategorized: bepridil, risperidone, terfenadine, chlorpromazine, clozapine, ranolazine, metoprolol, mexiletine, and loratadine. We investigated if chronic pacing of hiPSC-CMs improves proarrhythmia predictivity of the miscategorized drugs. hiPSC-CMs (iCell2 Fujifilm CDI) were cultured for a week and were subjected to stepwise increase of pacing frequency every 5 days at 1Hz, 1.5Hz, and 2Hz, respectively, using xCELLigence RTCA ePacer (Agilent technologies). 7 hours after termination of the pacing, cells were incubated with the 9 miscategorized drugs for 30 minutes, following the CiPA protocol as described in Blinova et al. Both the field potential and impedance signals were recorded using xCelligence RTCA CardioECR and were analyzed using CardioECR data analysis software (Agilent technologies). Chronic pacing improved the iCell2 response in 3 important ways compared to non-paced iCell2. First, the long-term electrical stimulation reduced the events of quiescence in the high doses of bepridil, risperidone, terfenadine, metoprolol, and mexiletine. Second, it enhanced the cell response as measured by FPD and IBD50 for high risk drugs such as bepridil, risperidone, and terfenadine, which in turn greatly contributes to the divergence of risk probability between intermediate and low risk drugs in the Blinova's 2018 model. In addition, variance of beating rhythm also separated the tested high-risk drugs from low risk drugs. In summary, varied physiological responses due to immaturity of hiPSC-CMs may hinder the predictive capacity of these cells for pro-arrhythmia. Our data shows that electrical stimulation improves proarrhythmia predictivity.

PS 3114 The Inhibitory Effect of Different Solvents on hERG Potassium Channel

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Inhibition of hERG (human ether-a-go-go related gene) current may cause QT interval prolongation, which may result in potentially fatal ventricular tachyarrhythmia called Torsade de Pointes. *In vitro* hERG assay is widely used as screening assay to evaluate the potential cardiac safety-concerns for lead compound optimization; GLP hERG assay in drug development has become routine in pharmaceutical industry, which is a critical element of safety pharmacology tests required for IND submission. Solvent is an easily neglected aspect that has significantly impact on results of hERG assay. Poor solubility may underestimate the impact of a compound on hERG channel and lead improperly judgement for potential utilization of a compound. Researchers have put much efforts in selecting different solvents in order to improve compound's solubility. However, the effects of these solvents on hERG channels are rarely reported and thus remain unclear. Our lab provides insight into the blocking characteristics of solvents itself on hERG channels, which hope to arouse more attention to judicious utilization of solvents. We investigated four different solvents, Acetone, DMSO, Tween 80, and Cyclodextrin to evaluate their impact on hERG current. The 0.3% (v/v) working solutions of Acetone, DMSO and Tween 80 were prepared respectively by diluting with extracellular solution, and cyclodextrin was directly dissolved in extracellular solution to obtain 1 mg/ml working solution. The manual patch clamp was used to detect the hERG current. There is no inhibitory effect on hERG current with 0.3% (v/v) DMSO and Acetone, the percentages of inhibition were -0.66% ± 3.29% and -3.74% ± 2.85%, respectively. However, both 0.3% (v/v) Tween 80 and 1 mg/ml Cyclodextrin exhibit inhibitory effects on hERG current with the percentages of inhibition of 66.28% ± 2.30% and 20.50% ± 2.27%, respectively. Treating with 0.3% (v/v) Acetone had shown no obviously impact on hERG current, but it did show slightly cytotoxicity over recording time.

3115 Engineered Cardiac Tissue as an *In Vitro* Platform to Evaluate the Role of Cardioprotective Mechanisms for Acute Toxic Study and Chronic Cardiac Remodeling and Dysfunction

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Engineered cardiac tissues (ECTs) serve as robust *in vitro* models to study human cardiac diseases including cardiac toxicity assays due to rapid structural and functional maturation. Advanced glycation end-products (AGEs), either endogenously formatted or through diet, are the proteins or lipids that become glycated as a result of exposure to sugars and found to be an important player in the development or worsening of many degenerative diseases, such as aging and diabetes. To date, the effect of AGEs on cardiomyocytes and underlying mechanisms remains not been fully illustrated; therefore, we generated 3D ECTs culture using neonatal mouse ventricular cells to determine the effect of AGEs on ECT toxicity and function under two serum conditions. We found: (1) ECT structural and functional maturation was similar in 10 % and 15% fetal bovine serum until 14 days after ECT was set up; (2) ECT was resistant to AGEs induced cytotoxic and functional changes under 15% serum, but not under 10% serum condition; (3) Under 10% serum condition, AGEs could induce cell necrotic death at 400 μg/ml (detected by medium Lactate dehydrogenase), apoptotic cell death at 200 μg/ml (detected by cleaved caspase 3), and no cytotoxic effect at the concentration of 150 μg/ml or less; (4) However, AGEs at 150 μg/ml could negatively impact ECT function in time-dependent manner (reduced beating rate and global function); (5) at the same dose AGEs also increased oxidative stress (increased HO-1 and Nrf2), inflammation (increased PAI-1 and TNF-α), fibrotic response (increased transcript expression of Collagen I-α1, Collagen III-α1, CTGF and FN1), hypertrophy (increased transcript expression of ANP, BNP and β-MHC) and autophagic disorder (increased p62) over time. Thus, neonatal murine ECTs can serve as a robust *in vitro* model as a platform to evaluate the role of cardioprotective mechanisms for acute toxic study and chronic cardiac remodeling and dysfunction, which will fill the gap between monolayer cell culture and *in vivo* animal models.

3116 TCDD Alters Dynamic DNA Methylation Patterns Crucial for Cardiomyocyte Maturation and Implicated in Disease

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Congenital heart disease (CHD) is the leading birth defect worldwide, yet the precise etiological factors underlying CHD remain largely unknown. Epidemiological studies estimate that <15% of cases of CHD can be traced to known Mendelian inheritance, suggesting that environmental agents may contribute to CHD during development. Exposure to tetrachlorodibenzo-p-dioxin (TCDD) in the young has been associated with disease conditions in adult fish, birds and mammals, including higher incidence of congenital heart abnormalities. TCDD is a potent ligand for the Ah receptor (AhR) - a sensor of environmental signals as well as morphogenic and detoxification pathways. Our lab has previously shown that AhR ablation or activation by TCDD disrupt molecular networks involved in heart formation and function. During development, the heart has to adapt to diverse physiological and pathophysiological needs. Several epigenetic processes have been implicated as modulators of cardiac gene expression in development and disease. Most recently, DNA methylation was shown to be highly dynamic during cardiomyocyte development, postnatal maturation, and disease. To test the hypothesis that TCDD exposure during cardiomyocyte development alters DNA methylation and gene expression patterns important in cardiomyocyte maturation, the stable pNkx2-5PuroIRES2eGFP cell line was established by differentiation from mouse embryonic stem (ES) cells. These cells express the selection markers puromycin resistance and eGFP under control of the Nkx2-5 promoter as well as markers characteristic of cardiomyocytes. These cells were treated with either 5nM TCDD or DMSO (control) after 24, 48, 72, and 96 hours of culture. Cells were harvested in duplicate after 24, 72, and 96 hours of treatment and compared to those treated for 24 hours with DMSO as well as ES cells. To assess genome-wide gene expression and DNA methylation, RNA- and methyl- seq analyses were performed with respect to the 24 hour DMSO control group. Our analyses showed significant correlation between expression and DNA methylation in 122 genes, many of which are involved in toxicological pathways related to cardiac transformation, cardiac hypoplasia, and cardiac damage. Our findings provide a key step towards elucidating the complex epigenetic patterns and mechanisms surrounding the development of congenital heart disease.

3117 Influence of Nanoparticle Inhalation on Cyclooxygenase-2 Metabolites during Gestation

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Nano-titanium dioxide (nano-TiO_2) is an engineered nanomaterial that is commonly incorporated in diverse advanced materials. We have reported that pulmonary nano-TiO_2 exposure has adverse systemic microvascular effects, but the mechanisms and impacts on reproduction are not well understood. Prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) are derivatives of arachidonic acid (AA) and are critical parts of cardiovascular and reproductive health. PGI_2 is an endothelium-dependent vasodilator, while TXA_2 is a platelet produced vasoconstrictor. Their balance is vital to maintain maternal/ fetal vascular function and adaptation throughout gestation. PGI_2 and TXA_2 reactivity within the microcirculation are fundamental components to maternal and fetal health. In this study, uterine microvascular PGI_2 and TXA_2 reactivity were assessed in uterine arterioles from virgin and pregnant rats after nano-TiO_2 inhalation exposures. Sprague-Dawley rats were either not exposed, naive (n=9) or sham- filtered air (n=9) or exposed to nano-TiO_2 aerosols (n=8) during gestation. Nano-TiO_2 aerosols were generated with Evonik- P25 (primary particle size= 21 nm and a zeta potential= -57 mV). Whole-body inhalation exposure (concentration= 12±0.5 mg/m^3) was performed for 6 hours/day for 6 days (cumulative lung burden= 525±16 μg). The aerosol count mean aerodynamic diameter (182±2 nm) was determined with a high resolution electrical low pressure impactor (ELPI). Rats were euthanized on gestational day 20 (24-hours post final inhalation exposure). Uterine microvascular reactivity was assessed via pressure myography. Naïve group characteristics: age 92±11 d, and mass 252±11 g. Sham-air group characteristics: age 87±2 d, and mass 268±8 g. Nano-TiO_2 group characteristics: age 70±5 d, mass 246±7 g, and MAP 67±9 mmHg. PGI_2 and TXA_2 responsiveness was recorded as diameter changes over increasing concentration ranges of AA derivatives. PGI_2 reactivity was not different between groups. However, TXA_2 reactivity was significantly decreased after nano-TiO_2 inhalation (percent change= 58±14%). Ongoing studies are investigating the impact of this potential deviation of TXA_2 and PGI_2 balance during gestation. *Support: NIH ES015022 (TRN); U54GM104942 (SH).*

3118 Effects of Long-Term Waterpipe Smoke Exposure on Atherosclerosis in ApoE Knockout Mice

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Waterpipe smoke (WPS), a type of tobacco smoke, is currently rising in popularity in the United States, specifically with respect to college-aged youths. Despite this alarming trend and waterpipe's centuries-long history, little is known about the long-term impact of chronic waterpipe smoke (WPS) on the development of atherosclerosis. Apolipoprotein E deficient (ApoE$^{-/-}$) mice have been shown to exhibit increased atherosclerotic plaque development in the brachiocephalic artery and the aortic arch. Furthermore, the atherosclerotic build-up in these hyperlipidemic animals appears to become exacerbated by exposure to ambient particulate matter and other forms of particulate pollutants such as cigarette smoke. Therefore, it is reasonable to postulate that other forms of tobacco smoke may also increase atherosclerotic plaque progression. Genetically modified male and female mice were exposed to either diluted WPS (46.46 ± 24.82 mg/m^3) or purified air via nose-only inhalation for 2 hours/day, 4 days/week, for 5 months. The brachiocephalic artery and aortic arch of all animals were excised and transversely sectioned and stained to identify lipid and collagen contents of the arteries. Animals exposed to WPS exhibited differences in the wall thickness and percent lesion intrusion into the arterial lumen compared to air controls indicating a greater degree of plaque formation. Lipid deposition and collagen buildup measured in the exposed cohort also differed compared to controls and provide insights into the compositional differences of plaques found in the different exposure groups. Quantitative assessment of differential staining furthers understanding of lesion severity and translates to the effect of WPS on the exacerbation of atherosclerosis. Previous studies have indicated that long-term inhalation of WPS alters the autonomic nervous system control of the heart by measuring heart rate variability. The current study indicates that WPS can influence the progression of cardiovascular disease in susceptible individuals. Taken together, these studies inform the cardiovascular health effects of WPS exposure in hyperlipidemic individuals.

3119 Development of Heart Block in ApoE (-/-) Mice Chronically Exposed to Waterpipe Smoke

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Tobacco smoke is an environmental toxicant that has been shown to exacerbate instances of atherosclerosis and CVD. Waterpipe smoke (WPS), a subset of tobacco smoke, is currently rising in popularity in the United States, specifically with respect to college-aged youths. Chronic exposures to WPS in mice have been shown to significantly enhance atherosclerotic biomarkers and plaque formation. However, a limited number of studies to date have examined the abnormalities in cardiac-specific endpoints, such as atrioventricular signaling which may result from WPS-exposure. This study aims to determine whether WPS exposure is associated with irregularities in atrioventricular signaling such as conduction blockages. Genetically modified male mice (n=5) were exposed nose-only to generated WPS (apple flavored) or purified air for 2 hours/day, 4 days/week, for 5 months. These animals were implanted I.P. with cardiac transducers allowing for electrocardiogram (ECG) collection. Waveforms of the mice were collected during the evenings following exposures and analyzed to detect alterations in ECG segments related to atrioventricular conduction (e.g. P-R interval duration, occurrence of non-conductive P-waves, and QRS interval duration). Heart rate variability in these animals was also measured during corresponding time periods using collected ECG signals. Animals chronically exposed to WPS exhibited altered electrical conduction through the atrial compartments of the heart compared to controls. The QRS interval duration in the exposed rodents also showed impairments compared to the air-exposed controls. Additionally, the exposed cohort experienced a statistically significant decrease in heart rate for six consecutive weeks during the first half of the study. Waterpipe usage has increased significantly in recent years. Determining cardiac malfunctions resulting from WPS inhalation is imperative for providing accurate health care to current and past users.

3120 Heart Rate Variation and Human Body Burdens Environmental Mixtures: Results from the Nituuchischaayihtitaau Aschii Multi-community Environment-and-Health Study in Eeyou Istchee

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Heart rate variability (HRV), the examination of beat-to-beat fluctuations in heart rate, is a measure of cardiac autonomic regulation. Unexposed persons generally show increased HRV compared to their contaminant exposed counterparts. While many exposure-based studies have examined the effects of air pollution on HRV, few have examined the association between contaminant body burdens, such as, persistent organic pollutants or metals, and HRV. None have examined these effects as a mixture. Using data collected from the Multi-Community Environment-and-Health Study in *Eeyou Istchee*, we assessed HRV in two time domain measures; root mean square of successive differences (RMSSD) and standard deviation of the N-N (R-R) intervals (SDNN); and in three frequency domains; high frequency (HF), low-frequency (LF), and very-low frequency (VLF). Subjects with any cardiovascular disease or stroke were excluded from analysis and only adults aged 19 to 79 were assessed (n=440). We first examined mixture effects of twenty one organic and metal contaminants using principal component analysis (PCA) followed by multivariable general linear regression, adjusting for age, sex, body mass index, smoking status, and kidney disease. We then chose the significant response variables of the previous analysis and performed a Bayesian kernel machine regression (BKMR) to examine individual contaminant contribution as well as entire mixture effects on HRV. Significant and negative associations were found between HRV and principal component axis 2, which highly and positively loaded for PBDE-47 and PBDE-153, and negatively loaded for nickel. The association was found for RMSSD and SDNN, but not HF, LF, or VLF. BKMR showed a mixture effect trend of decreasing RMSSD and SDNN as exposure concentration quantile increased. No single contaminant was, by itself, significantly and solely responsible for the overall negative trend observed in the BKMR analysis, but rather, several contaminants appear to work in concert to exert their effects on the cardiac autonomic regulation. Taken together, this analysis shows that PBDE-47 and some oragnochlorines may be driving the observed reductions in HRV while some contaminants such as nickel appear to temper this effect. The combined mixture effects of the contaminant is suggestive of a negative trend in both time domain measurements. This study expands our knowledge of the effects of environmental contaminant mixtures on HRV using novel statistical methods (PCA and BKMR).

3121 Methylmercury Accelerates Monocyte Adhesion to the Endothelial Cells by the Induction of Cytokine and Intercellular Adhesion Molecules

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The development of cardiovascular disease (CVD) has many facets ranging from unhealthy lifestyle choices to unavoidable environmental toxins. Among these toxins that contribute to CVD, environmental, and dietary exposure to organic mercury, in the form of methyl mercury (MeHg), presents a considerable cause for concern. While traditionally labeled a neurotoxin, MeHg has been epidemiologically linked to CVD pathologies; however, its role in development and promotion of atherosclerosis, an initial step in more immediately life-threatening CVDs, remains unclear. This study was conducted to examine the role that MeHg plays in the adhesion of circulating monocytes to vascular endothelial cells, a critical step in atherosclerosis, and attempts to clarify the underlying mechanisms. MeHg treatment significantly induced the adhesion of monocyte to Human microvascular endothelial cells (HMEC-1), while also upregulating the production of proinflammatory cytokines interleukin-6 (IL-6), interleukin-8 (IL-8). Further, MeHg treatment also upregulated the chemotactic cytokine monocyte chemoattractant protein-1 and intercellular adhesion molecule-1 (ICAM-1). Our results further demonstrated that MeHg stimulated a significant increase in NF-κB activation as measured by the eLUCIdate™ NF-κB reporter cell line. These findings suggest that NF-κB signaling pathway activation by MeHg is an important factor in the binding of monocytes to endothelial cells. This study provides new insights into the molecular actions of MeHg that can lead to endothelial dysfunction, inflammation and subsequent atherosclerotic development. The results of this study also contribute to our understanding of the detrimental effects of human exposure to MeHg which remains an important human health concern in a rapidly industrializing world.

3122 Electrophysiology Testing in Drug Development: Evaluation of Intracardiac Conduction Parameters in Dogs

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Atrial fibrillation (AF) affects about 3 million people in the US, 30% of whom experience no symptoms at all. AF is one example of a cardiac arrhythmia that can cause a disturbance in normal electrical conduction of the heart. Electrophysiology testing (ET) is routinely performed in human medicine to assess arrhythmias, such as AF, and identify any sites within the conduction system for blocks or delays. However, this common human technique is rarely performed during nonclinical drug development. The objective of this study was to evaluate the cardiac conduction times and effective refractory periods (ERP) from the sinus node through to the ventricles in male beagle dogs. Six dogs (10 to 12 kg) were anesthetized (isoflurane 2-5%) and intracardiac electrode catheters were placed, percutaneously, through the jugular veins (coronary ostium (CO) and high right atrium (HRA)) and femoral vein (HIS bundle and right ventricular apex) under fluoroscopic guidance. A surface ECG and femoral artery pressure catheter were also placed. ET was conducted in a passive (intrinsic rhythm) and dynamic phase (atrial and ventricular pacing). Continuous atrial pacing for 30 seconds at three cycle lengths (CL; 400, 330 and 300) was used to determined sinus node recovery time. ERPs of the atria, AV node and ventricles were assessed at the same CLs in a standard train of 8 impulses and 1 premature impulse at progressively shorter coupling intervals. The following parameters were measured; intra-atrial conduction time, PA interval; interatrial conduction time, HRA-left atrium or CO; HRA-HIS, AH; HIS-ventricles, HV; sinus node recovery time (SNRT) and SNRT corrected; and atrial, AV and ventricular ERPs. The SNRT was 790±284, 681±208, 639±161, SNRT corrected was 171±121, 166±110, 125±65, AH interval was 55±6, 60±3, 50±4, HV interval was 38±19, 58±19, 52±21, atrial ERP and AVERP were similar with values of 220±84, 141±25, 153±26, and VERP was 155±10, 150±6, 150±5. Values are presented for 400, 330 and 300 msec CLs, respectively, and are expressed as mean±SEM. Intracardiac placement of the electrode catheters and experience with conducting pacing studies are critical to the success of collecting intracardiac conduction times and effective refractory periods in dogs. The results of this study demonstrate both the comprehensive electrical evaluation of the heart and repeatability of the ET protocol for arrhythmia generation and safety evaluation of new chemical and biological entities.

3123 A Mouse Model of Doxorubicin-Induced Subclinical Cardiotoxicity

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Subclinical cardiotoxicity at low cumulative doxorubicin (DOX) doses can manifest into irreversible cardiomyopathy in some long-term cancer survivors. However, the underlying mechanisms are still less clear. To get better insights, echocardiographic assessment of cardiac function was performed in adult $B6C3F_1$ male mice at 1-, 4-, 10-, 17-, and 24-week after receiving 6, 9, 12, or 24 mg/kg cumulative DOX dose or saline to examine progression toward late-onset cardiotoxicity. In these mice, 24 mg/kg dose resulted in a significant decline in systolic function (Left Ventricular Ejection Fraction (LVEF) and Fractional Shortening (FS)) from 4- to 24-week after treatment compared to saline-treated counterparts. While there was no statistically significant effect elicited by lower cumulative doses (6 and 9 mg/kg) in systolic function at any time during the 24-week recovery, a significant time-dependent decline in both %LVEF and %FS was observed during the recovery period. At 24-week after 6 – 24 mg/kg total DOX, a significant dose-related decrease was observed in the transcript levels of ryanodine receptor 2 and sarco/endoplasmic reticulum calcium ATPase 2, with a significant 1.47- and 1.27-fold decline, respectively, at the highest cumulative dose. These genes encode proteins crucial in regulating intracellular calcium concentration within cardiomyocytes essential for contractility of cardiac muscle. These mice also showed a significant dose-related increasing trend in the total number of apoptotic cells with a significant 1.9- to 3.5-fold elevated level after exposure to 6 to 24 mg/kg total DOX dose compared to concurrent saline-treated controls. Altogether, subclinical cardiotoxicity in our mouse model was associated with molecular changes relating to calcium homeostasis and apoptosis even at low cumulative doses later in life. In long-term cancer survivors with underlying cardiovascular risk factors, such molecular changes in the heart may potentially lead to irreversible cardiac damage.

3124 Protective Role of Roflumilast against Cadmium-Induced Cardiotoxicity through Inhibition of Oxidative Stress and NF-κB Signaling in Rats

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Cadmium (Cd), a potent cardiotoxic environmental heavy metal, induces oxidative stress and membrane disturbances in cardiac myocytes. Phosphodiesterase (PDEs) retards the positive inotropic effects of β-adrenoceptor activation by decreasing levels of cAMP via degradation. Hence, PDE inhibitors sensitize the heart to catecholamine and are therefore, used as positive inotropic agents. The present study was designed to probe the potential attenuating effects of the selective PDE4 inhibitor (Roflumilast, ROF), on cardiac biomarkers, lipid profile, lipid peroxidation products, antioxidant status and histology of cardiac tissues against Cd-induced cardiotoxicity in rats. Rats were randomly distributed into four different groups: group 1, served as the normal control group. Group 2, served as the toxic control group and were administered Cd (3 mg/kg, i.p.) for 7 days. Groups 3 and 4, served as treatment groups that received Cd with concomitant oral administration of ROF doses (0.5 and 1.5 mg/kg), respectively for 7 days. Results showed Cd significantly increased the serum lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) levels by 333.24 ± 14 and 213.63 ± 5.8 (IU/L), respectively. Both ROF doses significantly reduced Cd-induced LDH and CPK levels in dose dependent manner. Also, ROF doses significantly reversed the Cd-induced TC, TG, LDL VLDL serum levels of 233.06 ± 8.05, 114.42 ± 2.35, 186.92 ± 8.68 and 22.88 ± 0.47 (mg/dl), respectively. The reduction of HDL serum level by Cd treatment (23.25 ± 1.22 mg/dl) was significantly counteracted by both ROF co-treatment to 29.10 ± 2.14 and 32.33 ± 1.47 mg/dl, respectively. Moreover, Cd causes a significant increase in myocardial malondialdehyde (MDA) contents and decrease in cardiac glutathione (GSH) level, superoxide dismutase (SOD) and catalase (CAT) enzyme activities. Whereas both doses of ROF, significantly reversed the Cd effects on these oxidative stress markers and antioxidant enzymes. Western blot analysis showed a significant induction of non-phosphorylated and phosphorylated form of NF-κB p65 expressions, and a significant reduction of glutathione-S-transferase (GST) and NQO1 expressions mediated by Cd treatment compared to control group in the heart tissue. These effects were significantly reversed by ROF treatments. Histopathological changes were also improved by ROF administration as compared to Cd treated rats alone. In conclusion, Roflumilast exhibited attenuating effect against Cd-induced cardiac toxicity.

3125 Determination of Blood Pressure Liabilities through Assessment of Vessel Reactivity Using a Tissue Myograph System

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Pharmacologically induced hypertensive and hypotensive disorders are leading causes of post-approval adverse vascular events. Chronic hypertension is associated with heart disease, arterial stiffening, stroke, kidney disease, and glomerular disease. Acute hypotension can cause dizziness, fainting, nausea, fatigue, and in severe cases; life threatening conditions such as oxygen deficiencies in the heart and brain or shock. Testing for potential blood pressure liabilities preclinically is required for IND approval of small molecules (ICH S7A) and is typically performed in parallel or following IND-enabling toxicology studies. Attrition at this stage in development is costly and can be detrimental to the success of a pharmaceutical development program. Screening for potential blood pressure effects early in drug development can help identify these liabilities and aid in candidate selection during lead optimization. Testing isolated rodent superior mesenteric (SM) arteries in the *ex vivo* myograph system provides an inexpensive method which allows early screening of direct pharmacologic effects on vessels. In this study, four vasoactive pharmaceutical agents were tested to assess vessel reactivity. The SM arteries were treated with escalating concentrations of epinephrine (0.01, 0.1, and 1 μM) and phenylephrine (0.01, 0.1, and 1 μM) to assess vasoconstriction. Vasodilation was assessed by treating these vessels to escalating concentrations of sodium nitroprusside (0.1, 1, and 10 μM) and minoxidil sulfate (0.05, 0.5, and 5 μM). Epinephrine and phenylephrine produced dose dependent responses, increasing tension at the highest concentrations by approximately 4- and 2-fold, respectively. Sodium nitroprusside and minoxidil sulfate produced dose dependent responses in pre-constricted vessels, decreasing tension at the highest concentrations by approximately 2-fold. To assess how these effects on the vasculature affect blood pressure *in vivo*, a single dose of epinephrine (4 μg/kg, i.v.), phenylephrine (50 mg/kg, oral), isosorbide mononitrate (250 mg/kg, oral), or minoxidil (10 mg/kg, orally) was administered to telemetered rats and blood pressure was monitored continuously. Epinephrine and phenylephrine caused a marked increase in mean arterial blood pressure. Isosorbide mononitrate and minoxidil caused a marked decrease in mean arterial blood pressure. This study demonstrates the ability of the myograph system to detect vessel reactivity *ex vivo* as well as the translatability to an *in vivo* model. Early screening for these potential blood pressure effect can aid in identifying liabilities and developing de-risking strategies.

3126 Establishing the Mode-of-Death of Combined Methamphetamine and Fentanyl by Leveraging an *In Vivo* Model for the Simultaneous Assessment of Diaphragmatic Force and Cardiovascular Parameters

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Over the past decade, traces of fentanyl (FENT) has been found, at alarming rates, in persons whose deaths have been attributable to cardiovascular collapse from methamphetamine (METH). FENT reduces, principally, respiratory drive that may lead to concomitant cardiovascular consequences. In theory, METH's stimulatory effect should protect against the depressant effects of FENT, therefore the cause of death (cardiovascular or respiratory) is puzzling and has yet to be established. Elucidating the combined cause of death is paramount for future preventative or therapeutic measures. Male Sprague Dawley anesthetized (2.5% isoflurane) rats were ventilated mechanically and instrumented to simultaneously assess left ventricular hemodynamics as well as diaphragmatic force. In this setting, the dose-dependent effects of FENT (5-40 ug/kg IV bolus) and METH (0.1-1 mg/kg IV bolus) were studied. METH alone, dose-dependently, increased force of diaphragmatic contractions 3 minutes post dose by 4, 18, and 27% with 0.1, 0.2, and 0.4 mg/kg and negligible effects on peak left ventricular pressure (LVPmax). Administration of 0.6 and 1.0 mg/kg elicited completed cardiovascular collapse and death. FENT alone transiently decreased diaphragmatic contractions (peak reduction of -65, -79, -85, and -94% and LVPmax (-22, -29, -33, and -38%) with 5, 10, 20, and 40 ug/kg. To assess the effect when administered in combination, FENT (10 ug/kg) was administered twice (with and without METH (0.4 mg/kg)) separated by a 1-hour washout period. FENT alone decreased force of diaphragmatic contractions by -63% and LVPmax by -19%. FENT+METH resulted in an unexpected greater reduction (-88%) in diaphragmatic force and similar changes in LVPmax. A similar study was performed to control for the administration FENT twice in an experiment. Contrast to FENT+METH, the second dose of FENT alone resulted in less of a respiratory depression compared to the first dose. Taken together, combinations of FENT and METH result in greater reduction in force of diaphragmatic contractions than FENT alone and

likely results in a greater chance of intoxication. This study demonstrates that potential *in vivo* cardiovascular and respiratory liabilities of opioids and other abused drugs can be assessed simultaneously.

PS 3127 A Rodent Model to Examine the Impact of Sleep on Cardiovascular Responses to Environmental Stressors

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Cardiovascular (CV) disease remains the preeminent cause of premature death worldwide. Among the contributors to CV disease, poor sleep is among the most common with more than 1/3 of Americans habitually sleeping suboptimal lengths of time necessary to promote health. Critically, perturbed sleep patterns (e.g., insomnia, short sleep duration) alter CV function and increase risk of CV diseases such as atherosclerosis, stroke, and hypertension. Apart from overt impacts, acute sleeplessness may also prime the CV system to increased sensitivity to environmental stressors in the short term, even in healthy people. For example, acute sleep loss has been linked to altered CV responses to subsequent mental stress and cold sensation in young, healthy people. Thus, this paradigm has the potential to reveal subtle impacts of sleeplessness including its impact on responsiveness to environmental stressors such as air pollution. While models of sleep loss have been proposed, a robust analysis of the CV impacts of one night of poor sleep has not been undertaken in a rodent model. To that end, we disrupted the sleep of rats by gentle handling for 5 s every 30 min for 5 h during the normal sleep cycle (09:00 - 14:00). CV function, including heart rate (HR), blood pressure (BP), the electrocardiogram, and core body temperature (T) was constantly monitored using implanted telemeters. Telemetry also allowed monitoring of locomotor activity, which was used as an indirect metric of sleep. Sleep disruption elevated activity levels during the disruption period relative to normal undisrupted sleep (~7x, p=0.0003), and caused activity levels that mirrored those of the preceding waking period. Importantly, sleep disruption also increased BP (systolic +7mmHg, p<0.0001; diastolic +4mmHg, p=0.005), HR (+27 bpm, p<0.0001), and T (+0.3^{0} C, p=0.0007), and decreased QA interval (-1.9 ms, p=0.0002), an inverse metric of cardiac contractility. Analysis of HR and BP variability, baroreceptor sensitivity, and systemic inflammation and stress are forthcoming. Given that nocturnal hypertension is a key driver in the long-term cardiovascular adversity associated with sleeplessness, this approach may prove useful in assessing the role of BP dysregulation in the interactive effects of sleeplessness and environmental factors. *This abstract does not reflect US EPA policy.*

PS 3128 Revisiting ICH S7B: Enabling Effective Clinical Evaluation Strategies Using a Science-Based Nonclinical *In Vivo* QT Model Based on Detection Sensitivity and Quality Criteria

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The ICH S7B guideline describes the non-clinical evaluation of human therapeutics for pro-arrhythmic risk, i.e. Torsades de Pointes (TdP). The two principal models highlighted in guidance are the *in vitro* I_{Kr} (hERG) and *in vivo* QT assays. The guideline has been invaluable in identifying and driving the use of these test systems to evaluate hERG blockade-mediated QTc prolongation, given that drugs known to cause TdP inhibit hERG function *in vitro* and prolong the QTc interval *in vivo*. A limitation of the guideline is the absence of any details on study design requirements, assay sensitivity, or the definition of quality, especially the QT analysis. It is possible that the absence of such detail may have led to an unintentional consequence: not leveraging the full value of the *in vivo* QT assay for informing the need for routine or extensive (ICH E14) QTc evaluation. We obtain statistical analyses on all cardiovascular assessment studies that inform on the quality the study performed. Our approach to performing cardiovascular telemetry studies in nonrodent species includes an evaluation of study quality, defined as the ability to detect a predefined magnitude of change in QTci (QT corrected with an individual animal heart rate correction factor). Using a retrospective power analysis of multiple studies (n=14 dog; n=6 NHP), we determined the mean power for a specific study design (N=8 per group) in both canines and cynomolgus monkey (NHP). The output of the power analysis is the minimal detectable effect at a specific probability level of 80%. The double Latin-square cross-over design provided an average sensitivity to detect a 2% (5 msec) change in QTci in canines and 2% (7 msec) in NHP. These findings indicate that this experimental approach has a consistent and reproducible sensitivity level that enables a robust QTci risk evaluation and inform the safe progression of new agents, e.g., create an integrated pro-arrhythmia risk assessment. The inclusion of power analysis (sensitivity) data in a regulatory submission provides key information to critical stakeholders about the quality of the *in vivo* QT assessment and value for human safety testing.

PS 3129 An *In Vitro* Human Population Model for Screening Environmental Chemicals for the Cardiotoxicity Hazard

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Adverse effects on the cardiovascular system are a major liability for pharmaceuticals, so the potential for cardiotoxicity is carefully assessed. However, environmental chemicals are seldom tested for their cardiotoxic potential, as no *in vivo* or *in vitro* cardiotoxicity tests are required for non-pharmaceutical compounds. Thus, with the exception of particulate matter and a few well-studied substances, the extent to which the chemicals in commerce and the environment may contribute to human cardiovascular disease morbidity is essentially unknown. Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes have become an important organotypic *in vitro* model for cardiotoxicity testing because they demonstrate biological relevance, high sensitivity and specificity, and consistent reproducibility of both baseline and treatment-induced characteristics. Importantly, iPSC-derived cardiomyocytes have been derived from multiple individuals and have previously demonstrated a reproducible and clinically-relevant model for *in vitro* testing of population responses to drugs and chemicals. Therefore, we hypothesized that a population-based model of iPSC-derived cardiomyocytes from a diverse set of normal human donors can be used to assess environmental chemicals for their potential cardiotoxicity hazard. To test this hypothesis, we conducted concentration-response screening of more than 1000 diverse chemicals (pharmaceuticals, industrial chemicals, pesticides, flame retardants, PAHs, and food constituents) in iPSC-derived cardiomyocytes from 5 humans that vary in their baseline and treatment-induced cardiophysiologic characteristics. We quantified kinetic calcium flux and high-content imaging data following chemical exposure, examined a panel of cardiophysiologic and cytotoxicity phenotypes, and calculated point-of-departure (POD) values for each chemical and donor. We observed chemical-specific variation in both potency and degree of population variability. These results show the potential for various environmental chemicals to affect the beating function of iPSC-derived cardiomyocytes. Ultimately, this study demonstrates the feasibility of using an organotypic population-based human *in vitro* model for rapid, high-throughput screening to quantitatively assess the cardiotoxic potential of chemicals for which little cardiotoxicity information is available.

PS 3130 Genotoxicity in the Heart-Brain Axis following Inhalation of Hexavalent Chromium [Cr(VI)] in a Rat Model

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As our global population continues to grow and live longer, diseases of heart and brain are becoming more prevalent. In order to better tackle the medical problems associated with aging bodies, we need better understanding about how our bodies are united. Decades of research have characterized the roles of individual organs in diseases and organ responses to chemical insults, but in most cases we are still lacking disease-modifying therapies. Here, we aim to bridge the traditional fields of neurotoxicology and cardiovascular toxicology to better understand how these two organs are physiologically connected. We are particularly focused on how DNA damage in both heart and brain contributes to systemic inflammation and associated diseases. We are using Cr(VI) as a model to induce DNA damage, given its known genotoxic effects. Cr(VI) is a known human carcinogen and listed among the Top 25 environmental pollutants by the Environmental Protection Agency. Most research investigating the toxic effects of Cr(VI) focus on known target organs (e.g. lungs, liver, kidneys), though there is a growing body of literature that describes the toxic effects in the heart and brain. Rodents studies demonstrate that up to 46% of inhaled of Cr(VI) can reach the brain and induces widespread neurodegeneration, though it is unclear how this may contribute to neurological diseases. Several human studies have linked occupational Cr(VI) exposure or chromium metallosis with neuropsychological disorders and brain tumors. Hence, there is a need to better understand how Cr(VI) affects heart and brain individually, as well as effects on their interconnected health. Our comet assay shows increases in DNA damage in the heart and different regions of the rats exposed to Cr(VI), with different dose-response profiles among organs and regions as well as sexes; therefore, we aim to evaluate how Cr(VI) inhalation accumulates in heart and brain tissues, induces DNA damage and repair pathways, and contributes to systemic inflammation. *This work was supported by NIEHS*

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3131 Predicting Putative Cardiotoxic Chemicals Using Tox21 qHTS Data

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Cardiovascular (CV) disease is the leading cause of death for people of most ethnicities in the United States and is a major source of pharmaceutical drug attrition. A potentially significant but underappreciated risk factor contributing to the development and severity of CV disease is exposure to bioactive substances in our environment. The heart and vascular system have been shown to be vulnerable to several environmental agents such as pesticides, flame retardants, polycyclic aromatic hydrocarbons (PAHs), plasticizers, ambient air pollution and metals. There is an urgent need to evolve the predictive toxicological testing paradigm to identify environmental chemicals that might be contributing to CV disease. In this study, we are designing a strategic paradigm to screen for potential environmental chemical cardiotoxicity that may also help in preclinical testing to identify pharmaceutical CV safety liabilities. To screen compounds for potential CV effects, we are leveraging *in silico* tools and *in vitro* high-throughput screening (HTS) data. The US federal consortium on toxicology in the 21st century (Tox21) providing broad chemical coverage, and the EPA toxicity forecaster program (ToxCast) providing broad biological coverage, produce quantitative HTS data on thousands of chemicals across a wide range of assays covering critical biological targets and cellular pathways. Using these data, we have designed a CardioToxPi, which is a visualization tool and a ranking system in which we have prioritized chemicals by bioactivity score against molecular and cellular targets known to mediate bioactivity in the CV system. We are working to generate *in silico* predictive models that can provide predictions of CV toxic events across large numbers of chemicals. The computational toxicology methodology applied here aims to include diverse data streams, toxicity mechanisms & pathways, and derive thresholds that could be applied to specific decision contexts. This method will be applied to screen and prioritize chemicals with constrained or no toxicity information for further assessment.

3132 Perfluorobutanoic Acid (PFBA) Affects Larval Weight Changes in the Beet Armyworm *Spodoptera exigua*

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Toxicological studies on long chained perfluorinated compounds (PFCs) in a number of organisms suggest that they are endocrine disruptors. Perfluorobutanoic acid (PFBA), one of the replacement compounds for long chained PFCs, have been shown to accumulate in plants. To evaluate the endocrine disruptive potential of PFBA on the development and plant interaction of insect pests, its dietary effect on the larval weight of the Beet armyworm, *Spodoptera exigua* was investigated. Second instar larvae were fed with PFBA spiked artificial diets and leaves from soybean plants grown on soils spiked with PFBA solution. Spiked PFBA concentrations were ~200 ug/kg and 10 mg/kg respectively on the artificial diets and the soils. The artificial diet larval weight experiment was observed until the larvae commenced pre-pupal inactivity while the soybean plant leaf diet exposure study was observed for 3 days. For the artificial diet study, the treatment groups showed consistently increased weight over the Controls from 1 - 7 days post treatment (dpt). The ratio of weight gain of this treatment group over the Controls ranged from 1.08 to 1.39. The differences in weight gain were significant from 3-7 dpt ($p < 0.05$). The study involving the soybean leaf diet showed increased larval weight of the exposed over the controls for all three days with the ratio of weight gain ranging from 2.53 to 5.71. The leaf morphology showed damage of 27.01±2.45 and 22.52±2.26 % by the Beet armyworm for the PFBA treatments and controls respectively at 2dpt. Taken together, the data suggests that the PFBA perturbed the weight gain of the exposed larvae as they transited from the second to fifth instar. The implications of these findings are the possibilities of plants' uptake of this PFC influencing the development and the plant damage potential of the Beet armyworm. Further studies are ongoing to understand the dose response relationship with respect to weight changes. Correlation of weight changes with transcriptomic changes is also being carried out to gain better insight into the findings here reported.

3133 Exposure to Perfluoroalkyl Substances (PFAS) in the Placenta and Altered Expression of Epigenetic Machinery-Associated Genes

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Per- and poly-fluoroalkyl (PFAS) substances are chemicals that are found in a variety of products including nonstick pans, cleaning supplies, fire-fighting foams, and water-repellent fabrics. While perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfate (PFOS) are no longer used in the United States, their persistence continues to be an environmental and health concern. In addition, a replacement PFAS, 2,3,3,3-tetrafluoro-2- (hepta fluoropropoxy) propanoate (GenX) is also present in waterways and air emissions as an environmental toxin. There is supportive evidence that exposure to PFAS results in adverse human health outcomes such as preeclampsia and reduced infant birth weight. Studies have also shown an association between PFAS including PFOA to epigenetic changes including hypomethylation in cord blood. However; the effects of perfluoroalkyl substances (PFAS) on the expression of genes that play a role in epigenetic regulation in the placenta are poorly understood. This study seeks to determine whether the expression of genes that play a role in epigenetic regulation (epigenetic machinery genes; EMGs) are altered in the placenta with PFAS exposure. Specifically, *in vitro* trophoblast JEG-3 cells were treated with PFOA, PFOS, and Genx (10-100 ng/mL), in serum or serum free media. A total of 96 EMGs involved in histone phosphorylation, ubiquitination, CpG methylation, deacetylation, acetyltransferases, and methyltransferases were assessed for mRNA expression changes through PT-PCR. Western blots were used to assess changes in protein expression in relation to PFAS treatments. The results revealed significant alterations after PFAS treatments in the expression of EMGs, including Aurora A kinase, *AURKA,* and Protein Arginine Methyltransferase 3, *PRMT3.* The results suggest that exposure to PFAS in the placenta is associated with the altered expression of key genes that play a role in epigenetic regulation in JEG-3 cells, specifically related to histone phosphorylation, demethylation, and deacetylation. There is a need for additional research to determine long-term human health effects due to PFAS exposure and the role of EMGs.

3134 Hexafluoropropylene Oxide Dimer Acid (HFPO-DA): GenX Induces Apoptosis in Liver Cells

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Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-proponate (Hexafluoropropylene oxide dimer acid, HFPO-DA) is a type of perfluorinated alkyl substances (PFASs), also known as GenX. PFASs are nonvolatile substances that are easily released into the environment during manufacture and use, so they are easily implemented in drinking water, soil, and air. GenX, unlike other PFASs, has a relatively short carbon length and has recently been used as a new alternative because of its ability to be released into the body. However, it has recently been detected in water sources in many countries, including the USA. The mechanisms and human intoxication directly attributed to GenX have not been elucidated. Therefore, the aim of the study was to investigate the apoptotic potential of GenX in human liver cells, HepG2. HepG2 cell was exposed to GenX (40-500 μg/mL) for 48 h showed a decrease in cell viability and significantly increased apoptosis rate. GenX was also induced oxidative stress in a dose-dependent manner indicated by increasing the production of intracellular reactive oxidative species (ROS). Quantitative real-time PCR results showed the mRNA expressions of apoptosis markers were up-regulated in the GenX-treated group. Compared with the untreated control group, mRNA expression level of BAX, caspase-3, caspase-9, and p53 was increased by 3.1, 1.2, 1.4, and 2.5-fold in the 500 μg/mL-treated group, respectively. In addition, similar tendencies were observed in protein expression level of apoptosis-related factors through western blotting. This study suggests that GenX induces apoptosis through oxidative stress in liver cells.

3135 A Comparison of PFAS Serum Concentrations in the General Population to Points of Departure Used in Regulatory Guidance

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Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals of growing concern to regulatory agencies. In deriving guidance values for PFAS in drinking water, agencies typically use a rodent serum PFAS concentration associated with a key toxicological effect as the point of departure (POD) to

estimate a human-equivalent dose. A comparison of agency PODs with serum concentrations in the general population would provide perspective on the toxicological significance of the exposures of the general population to PFAS. We first conducted an analysis of the serum concentration PODs from both national and state agencies with finalized drinking water health-based values for perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), perfluorodecanoic acid (PFDA), and perfluorobutane sulfonic acid (PFBS). Using PFAS serum concentrations measured in the most recent NHANES, we then conducted a margin of exposure (MOE) analysis to compare POD serum concentrations with those of both the geometric mean and 95th percentile of adults and children in the general US population. Agencies' PODs varied greatly for any given PFAS, and this was due to differences in selection of key studies, key toxicological effects, and model assumptions. For example, US EPA and the Minnesota Department of Environmental Protection estimated the highest POD as 38,000 ng/mL PFOA, whereas the New Jersey Department of Environmental Protection and New Hampshire Department of Environmental Sciences estimated the lowest POD as 4,351 ng/mL PFOA. MOE values also varied greatly depending on the agency and PFAS. For example, MOEs for the general population with PFOA serum concentrations at the 95th percentile (4.17 ng/mL) and geometric mean (1.56 ng/mL) ranged from 1,000-9,100 and 2,800-24,000, respectively. Similarly, MOEs for children aged 3-11 years in the general population with PFOA serum concentrations at the 95th percentile (4.19 ng/mL) and geometric mean (1.92 ng/mL) ranged from 1,000-9,100 and 2,300-20,000, respectively. Our analysis underscores the uncertainties in evaluations of PFAS toxicity. It further shows that current serum levels in the general population are well below any exposures that could potentially be associated with health effects.

3136 Computational Association of PFAS Exposure and Hypothyroidism

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Perfluoroalkyl substances (PFAS) are a group of man-made chemicals that are known contaminates of drinking water, particularly in communities surrounding military bases. Some studies, such as Project Viva, a longitudinal Prebirth Cohort, have concluded that exposure to some PFAS influence and may disrupt thyroid function in both mothers and infants. This study investigates whether there is an association between PFAS exposure and thyroid dysregulation using a systems toxicology approach to identify a probable molecular initiating event (MIE) in a proposed adverse outcome pathway (AOP). In addition, the populations most susceptible to thyroid dysregulation when exposed to PFAS in drinking water were proposed. Various computer programs, including online databases, data networks, biomonitoring data, and the literature were used to establish proposed chemical-gene-disease associations. The iodothyronine deiodinase 3 (DIO3) gene, which catalyzes the inactivation of triiodothyronine (T3) and thyroxine (T4), was of primary interest because it affects two thyroid hormones that are crucial in maintaining the body's metabolism. The proposed MIE suggests that PFAS bind to the DIO3 nuclear receptor, increasing the gene expression of DIO3. This leads to the deactivation of T3 and T4, ultimately resulting in hypothyroidism. Additionally, to determine if those who have autoimmune diseases are more susceptible to hypothyroidism when exposed to PFAS, this study examined the connection between Hashimoto's Disease and exposure to PFAS. Hashimoto's Disease is caused by the increased production of antibodies against thyroid peroxidase (TPO), an enzyme normally found in the thyroid gland that plays an important role in the production of thyroid hormones T3 and T4. Research suggests that an increase in transforming growth factor beta (TGFB) also inhibits TPO mRNA and protein expression in humans, and over stimulates DIO3. PFOS and PFOA are hypothesized to decrease TPO by increasing TGFB, further increasing the activity of DIO3 in patients with Hashimoto's. The results of this study suggest that there is a connection between PFAS and hypothyroidism, and that those who already have Hashimoto's Disease are even more susceptible to PFAS exposure.

3137 Proteomic Analysis Reveals Perfluoro-(3,5,7,9-tetraoxadecanoic) Acid (PFO4DA) Induced Hepatotoxicity via Activation of PPARs in Male Mice

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Perfluoropolyether carboxylic acids (PFECAs, $CF_3(OCF_2)_nCOO-$, n = 2-5), including perfluoro (3,5,7,9-tetraoxadecanoic) acid (PFO4DA), are emerging per- and polyfluoroalkyl substances (PFASs). PFO4DA has been recently detected in human blood samples in US and the environmental matrix. However, their potential toxicities for humans are unclear. In this study, we assess the hepatotoxicity of PFO4DA on male mice, especially its effects on liver lipid metabolism. Male mice were exposed to 0, 0.4, 2, or 10 mg/kg/d PFO4DA continuously for 28 d. We first measured and compared the liver weight, relative liver weight and serum biochemical parameters that indicating acute liver injury. Compared to control group, changes in liver injury index in 0.4 and 2 mg/kg/d PFO4DA groups did not exceed 20%. After 10 mg/kg/d PFO4DA exposure, relative liver weight and liver injury index including alanine aminotransferase, glutamic oxaloacetylase and alkaline phosphatase were significantly increased, suggesting acute liver injury. Liver and serum lipid content were then detected to assess the effects of PFO4DA on lipid metabolism. No change in triglyceride (TG) and total cholesterol (TCHO) content was observed in both three exposed groups, while in the liver of 10 mg/kg/d PFO4DA exposed mice, TG and TCHO levels were significantly decreased. Furthermore, differentially expressed liver proteins (DEPs) between the control and 10 mg/kg/d groups were identified using isobaric tags for relative and absolute quantification (iTRAQ) and bioinformatics analysis to explore the possible mechanism for PFO4DA toxicity. Western blotting was performed to verify iTRAQ results and analyze the expression levels of key proteins and nuclear receptors. Results showed that exposure to 10 mg/kg/d of PFO4DA led to 198 DEPs (56 down-regulated, 142 up-regulated) that were mainly involved in fatty acid metabolism, fatty acid degradation, peroxisome, and the PPAR signaling pathway, highlighting urea cycle disorder and stimulation of lipid metabolism in mice liver. The significantly increased PPARα and the downstream proteins after 10 mg/kg/d PFO4DA exposure might be mainly responsible for the decreased lipid content in liver. PFO4DA exposure could cause hepatotoxicity and decreased lipid content in mice liver, with PPAR pathway activation being a significant contributor to the observed toxicity. Our data implied that PFO4DA may not be a suitable alternative to PFOA. Considering that PFO4DA has been detected at much higher concentrations than PFOA in raw water samples, efforts to remove or at least decrease its occurrence in drinking water should be made urgently.

3138 Perfluorinated Compounds Decrease Expression of a Key Steroidogenic Enzyme in Leydig Cells

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Background: Perfluorinated compounds (PFCs) are synthetic chemicals, which were once widely used for industrial purposes. Because of their extraordinary resistance to degradation, PFCs are universally detected in environmental media, as well as in humans and wild life. As a result, environmental exposure to PFCs and their accumulation in human body present toxicological concerns. Over the past several decades, male reproductive functions have been declining in human and wildlife. The present study aims to investigate whether exposure to PFCs adversely impact the functions of male reproductive system. Hypothesis: Exposure to PFCs interferes with the expression of key steroidogenic enzymes in Leydig cells, which then results in a reduction in testosterone production. Method: A panel of four most commonly detected PFCs (PFDA, PFNA, PFOA and PFOS) were screened on three mouse Leydig cell lines (MLTC-1, MA10, and TM3) for interference on the expression of key enzymes involved in testicular steroidogenesis. Results: Perfluorinated carboxylates (PFDA, PFNA, and PFOA) at a concentration of 10 micromolar decreased expression of hydroxysteroid dehydrogenase 3 beta 6 (Hsd3b6) more than 50% in MLTC-1 cells but perfluorooctane sulfonate (PFOS) did not. Using PFDA as a representative of the perfluorinated carboxylates, we showed that PFDA similarly decreased Hsd3b6 expression (≥ 50%) in two additional Leydig cell lines (MA10 and TM3). PFOS decreased Hsd3b6 expression only in TM3 cells but not in MA10 cells. Concentration-response studies of PFDA (0.1-100 micromolar) in all three Leydig cell lines revealed that higher concentration of PFDA caused greater reduction in Hsd3b6 expression. In contrast, concentration-response study of PFOS in MLTC-1 cells showed an irregular concentration-response curve with reduction in Hsd3b6 expression only observed at the highest concentration tested. Conclusions: PFCs decreased the expression of Hsd3b6, which is a conserved and key enzyme, in male gonadal steroidogenesis. In comparison to PFOS, the three perfluorinated carboxylates (PFDA, PFNA, and PFOA) seem to have greater impact on Hsd3b6 expression at lower concentrations and exhibit consistent concentration-response effect in Leydig cells.

3139 A Model Template Approach for Rapid Evaluation of PBPK Models for Use in Human Health Risk Assessments

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Physiologically based pharmacokinetic (PBPK) models can significantly reduce uncertainty in components of a human health risk assessment (e.g., dose extrapolation across species) but evaluating PBPK models to meet quality assurance standards can be a slow process, particularly when model code is not readily available. In order to allow for faster evaluation of PBPK models, and thus faster evaluation of chemicals for human health risk assessments, we developed a model "template" that allows one to quickly implement various chemical-specific PBPK models. To test this approach, we created a template PBPK model applicable to multiple per- and polyfluoroalkyl substances (PFAS). This PFAS PBPK model template allows for representation of models with varying numbers of tissue compartments, oral and intravenous exposures, and multiple elimination pathways, including urinary, biliary, and fecal routes. The template also includes an option for renal resorption, which impacts clearance rates for some PFAS. We parameterized the PFAS template model using values from published PBPK models for perfluorohexanesulfonate (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA). We first verified that the template reproduced the published model results, then attempted to validate the model using independent data sets not used to calibrate model parameters. Following this template approach, only one primary model file needed to undergo quality assurance review. Subsequent model evaluation for individual PFAS only required the input files containing model-specific parameters to be reviewed. This greatly reduced the amount of time necessary to review the PBPK models for the three PFAS we evaluated. This template will allow for faster evaluation of PBPK models for use in assessing the potential human health effects of PFAS exposure and a more efficient quality assurance process, and it demonstrates a generalizable approach that could be applied to broader classes of chemical-specific PBPK models.

3140 *In Vitro* Binding of Human and Rat PPAR Alpha, Beta/Delta, and Gamma Receptors to PFAS, Fatty Acids, and Clofibric Acid

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The molecular mechanism by which some per- and polyfluorinated alkyl substances (PFAS) exert toxicity has largely been attributed to activation of peroxisome proliferator-activated receptor alpha (PPARα). Less attention has been given to PPAR beta/delta (PPARβ/δ) or PPAR gamma (PPARγ). Further, it is unknown how, or if, PFAS activation of PPAR paralogs differs between rats and humans. We utilized *in vitro* luciferase reporter assays with either human or rat PPARα, β/δ, or γ ligand binding domains hybridized with a Gal4 DNA binding domain to evaluate 9 PFAS (hexafluoropropylene oxide-dimer acid ammonium salt (GenXAS) and free acid (GenXFA), nafion byproduct 2 (NBP2), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorobutane sulfonate (PFBS), perfluorononanoic acid (PFNA), 6:2 fluorotelomer alcohol (6:2 FTOH), and perfluoromethoxyacetic acid (PFMOAA)), 3 endogenous fatty acids (oleic, linoleic, and octanoic), and the drug metabolite clofibric acid at 30 µM -1 mM. We also tested all chemicals for estrogen (ER), androgen (AR), and glucocorticoid receptor (GR) transcriptional activation using previously established stable transfection (ER) and viral transduction (AR, GR) luciferase reporter assays. All compounds, except 6:2 FTOH, activated both PPARα and PPARγ in both human and rat. EC_{10} values were calculated due to low fold induction for several compounds. Oleic and linoleic acid were the most potent human PPARα activators, while PFMOAA had the lowest EC_{10} and PFOS had the lowest fold induction (13% of max). For rat PPARα, GenX was the most potent activator (no difference between GenXFA and GenXAS), while NBP2, PFMOAA, PFBS, and PFOS all had very low fold induction (≤6% of max). For PPARγ, all compounds had similar potency in the human and rat receptor assays (log EC_{10} ranges: -3.33 - -3.86 versus -3.07 - -3.80), except for PFMOAA which only reached 3% and 1% of max fold induction, respectively. In contrast, the only compounds that activated both human and rat PPARβ/δ were oleic and linoleic acid; however, NBP2 weakly activated (~10% of max) rat PPARβ/δ at 300 µM. No compounds displayed *in vitro* AR or GR transcriptional activation. Only 6:2 FTOH exhibited weak ER agonism. Overall, all PFAS studied, except 6:2 FTOH, displayed both human and rat PPARα and γ activity, with some having similar potency to endogenous free fatty acids. Thus, it is likely that both PPARα and γ activation contribute to the adverse *in vivo* effects observed for PFAS. *Abstract does not necessarily reflect US EPA policy.*

3141 Comparative Evaluation of Mouse Mammary Gland Development and Pathways of Gene Expression following Prenatal Exposure to PFOA and GenX

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Legacy per- and polyfluoroalkyl substances (PFAS) are a group of contaminants known to be highly persistent in the environment and in humans. Previous studies have shown that gestational exposure to perfluorooctanoic acid (PFOA) impairs development of the female mouse mammary gland. The purpose of this study was to compare changes in mammary gland morphology and pathways of gene expression from *in utero* exposure to PFOA and a replacement PFAS, GenX (ammonium perfluoro-2-methyl-3-oxahexanoate), which is thought to exhibit more rapid elimination. Time-pregnant CD-1 mice were dosed with PFOA (0.l, 1.0, or 5.0 mg/kg) or GenX (0.2, 1.0, 2.0, or 10.0 mg/kg) from gestation day 1.5 to 17.5. Mammary glands were collected from offspring at weaning (PND 21 or 22) or during puberty (PND 36). Developmental scores between 1 and 4 were assigned to female and male mammary gland whole mounts by three evaluators blinded to treatment, based on a set of criteria for normal development at weaning or puberty. RNA was isolated from PND 22 female glands and changes in gene expression were assessed with an Affymetrix Mouse Clariom D transcriptome array and Ingenuity Pathway Analysis. Histopathology and internal dosimetry are underway. There were significant dose-dependent reductions in developmental score for female glands exposed to PFOA and GenX ($p<0.01$) and male glands exposed to GenX ($p<0.05$) (Jonckheere-Terpstra test). All female dose groups except 0.2 mg/kg GenX had mean scores significantly lower than control glands but no male dose groups differed from the control (Dunn's test). Preliminary evidence indicates 40 genes that are similarly changed (vs control) by PFOA and GenX, and a much larger number that exhibited treatment specific regulation. Among commonly regulated genes are those involved in insulin receptor, growth factor, PPARα, and p38 MAPK signaling as well as regulation of adipose browning and the epithelial mesenchymal transition. Altered expression of these pathways suggests dysregulation in the stromal portion of the gland may be responsible for delayed development of mammary epithelial tissue. Together, these data indicate that, despite more rapid elimination of GenX, both chemicals induce sex-specific impairment of female mammary gland development that may be modulated by prenatally-triggered changes in gene expression.

3142 Perflurooctane Sulfonate (PFOS) Alters Gut Microbiota Composition and Function in Mice

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Perfluorooctane sulfonate (PFOS) is a persistent environmental chemical that is toxic to human endocrine, reproductive, and immune systems. Toxicity can be mediated by multiple mechanisms. Recently, the gut microbiome has been reported to alter the fate and activity of environmental chemicals on the host. However, whether PFOS directly influences the gut microbiome community structure and function remains unknown. In this study, 6-8 weeks old male C57BL/6J mice were fed a control diet with and without 0.003%, 0.006% or 0.012% PFOS. 16S rRNA gene sequencing of cecal contents revealed that PFOS caused marked changes in the gut microbiota composition relative to control. For example, there was a significant increase in the relative abundance of Bacteroidetes ($FC=1.36$, $p<0.05$) and significant decrease in the relative abundance of Firmicutes ($FC=1.34$, $p<0.05$). Further, flow cytometry and ^{1}H NMR-based metabolomic profiling of cecal contents revealed the direct effects of PFOS on the gut microbiome metabolism and physiology. PFOS caused a significant dose-dependent decrease in CFDA (a proxy for metabolic activity) stained bacteria (63% ± 3.27 to 26.4% ± 0.87%, $p<0.0001$) indicating decreased metabolic activity of bacteria. ^{1}H NMR metabolic profiling revealed a significant increase in the uracil ($FC=1.6$), inosine ($FC=2$), hypoxanthine ($FC=2.14$), xanthine ($FC=1.5$), and uridine diphosphate ($FC=2$) after PFOS treatment. Overall, PFOS altered the gut microbiome composition and its function. These findings provide new insights and understanding of the mechanisms that may modulate the effects of PFOS and suggest new mechanisms to understand PFOS toxicity.

3143 Development of Gestational and Lactational Physiologically Based Pharmacokinetic (PBPK) Model for Perfluorooctane Sulfonate (PFOS) in Rats and Humans within a Bayesian Framework to Derive Health-Based Toxicity Values

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Perinatal exposure to perfluorooctane sulfonate (PFOS) has been associated with developmental toxicity in animal and human studies. However, risk assessment and derivation of health-based toxicity values for PFOS are conflicted due to the lack of tools for species extrapolation underling the toxicokinetics of gestational and lactational transfer and tissue distribution. To address this challenge, this study aimed to develop a gestational and lactational PBPK model in rats and humans to derive the human equivalent dose (HED) and reference dose for PFOS. Available animal toxicokinetic and human biomonitoring data were used for model calibration and optimization, and independent datasets were used for model evaluation. A Bayesian framework with Markov chain Monte Carlo (MCMC) simulation was applied to improve model performance and to characterize the variabilities and uncertainties of chemical-specific parameters during pregnancy and lactation. Model simulations were within two-fold of observed PFOS concentrations in fetal, neonatal, and maternal plasma. Estimated HEDs based on selected critical toxicity studies in rats following US EPA's guidelines ranged from 0.053 to 0.060 μg/kg/day, which are lower than the HEDs estimated by US EPA (1.6 μg/kg/day). Our results suggest that the derivation of health-based toxicity values based on developmental toxicity study should consider species differences in gestational/lactational dosimetry. This study provides insights into a complete risk characterization of PFOS and may help regulatory agencies in the reevaluation of PFOS risk in sensitive subpopulations, including fetuses, neonates, pregnant and lactating women. The validated gestational and lactational PBPK model can be used to improve risk assessment for other per- and polyfluoroalkyl substances (PFAS).

3144 *In Vivo* Toxicological Investigation of Per- and Polyfluoroalkyl Substances (PFAS) to Elucidate Structure-Bioactivity Relationships and Investigate Modes of Action

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Per- and polyfluoroalkyl substances (PFAS) are persistent compounds frequently detected in the environment, biota, and humans. In recent years, toxicological studies have addressed a wider range of PFAS, however, there are still thousands yet to be assessed. To investigate PFAS toxicity, we are conducting *in vivo* testing to better understand structure-activity relationships and to discern mechanisms of action. We developed a high-throughput screening (HTS) platform using early life stage zebrafish to evaluate the biological activity of individual PFAS of a variety of chain lengths and functional head groups. Initial investigation has focused on the carboxylate class. We identified and acquired a complete chain length series of PFAS with a single carboxylic acid head group and fully fluorinated chains from 4 (perfluorobutanoic acid) to 14 carbons (perfluorotetradecanoic acid). The purities of acquired PFAS were characterized and used to make stock solutions in dimethyl sulfoxide. Dechorionated embryos were statically exposed at 6 hours post fertilization (hpf) in individual wells of 96-well plates. We measured 22 morphological and 2 behavioral endpoints at 24 and 120 hpf. For each member of the carboxylate class (11 PFAS), we first conducted a preliminary concentration-response study, using a broad concentration range (0-100 μM, n= 12) to define the bioactivity of each PFAS. For each PFAS, we completed subsequent definitive testing with 36 fish at each concentration to calculate the EC_{10}, EC_{50}, EC_{80}, etc. We are using these concentration-response curves to investigate associations between PFAS carbon chain length and resulting biological activity. To begin to identify the mode of action of the carboxylate class PFAS, we also exposed embryos to the respective EC_{80} and conducted full-genome RNA sequencing using RNA collected from 48 hpf larvae. Collectively, through *in vivo* screening, structure-activity analysis, and transcriptomics, we aim to collect sufficient empirical data to allow us to predict the toxicity of the carboxylate class PFAS. Future work will utilize the outlined experimental design to investigate the sulfonate, sulfonamide, and telomer sulfonate PFAS categories.

3145 Gene Expression Changes Associated with Environmentally Relevant Doses of PFAS in an *In Vitro* Placental Trophoblast Model

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Per- and poly-fluoroalkyl substances (PFAS) are a group of chemicals that have been widely used in stain repellents, paints, polishes, protective coatings, and firefighting foams. While some of these chemicals, including perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been discontinued in the United States, their persistence in the environment and continued use internationally make them a toxicological concern. Moreover, GenX, a modern replacement for PFOA, has recently contaminated drinking water in the Cape Fear River in the Wilmington, NC area, warranting immediate analysis of the associated risks. While prior reports have shown that PFAS can cross the placenta, there is little understanding of how this class of compounds can impact placental function itself. Therefore, this study aimed at understanding the effects of PFAS exposure on placental gene expression and ultimately placental function. HTR-8/SVneos, an *in vitro* trophoblast cell line, were treated with an environmentally-relevant dose (10 ng/ml) of PFOS, PFOA, or GenX for 24 hours and mRNA/whole transcriptome sequencing was performed. To evaluate changes in gene expression profiles associated with PFAS exposure, genome-wide RNA sequencing count data were processed and statistically assessed. Genes showing differential expression associated with each PFAS exposure were prioritized based statistical significance. Pathway analysis on resulting genes revealed both PFOS and GenX induce changes in the expression levels of similar gene sets. Specifically, impacted pathways included cell movement and apoptosis with altered genes including but not limited to Tumor Necrosis Factor (*TNF*), Thiosulfate Sulfurtransferase (*TST*), Matrix Metallopeptidase 9 (*MMP9*), Nuclear Factor Erythroid 2-Related Factor 2 (*NFE2L2*), and Insulin Like Growth Factor 1 Receptor (*IGF1R*). PFOA, on the other hand, impacted different gene sets with dysregulation of artery and vein development signaling, most significantly Ephrin Type-B Receptor 2 (*EPHB2*). Several of these genes including but not limited to *MMP9*, *NFE2L2*, and *EPHB2* have published relationships to dysregulated signaling observed in preeclamptic placentas. Ultimately, this is among the first studies to look at whole genome PFAS impact on placental signaling at environmentally-relevant levels and highlights the need for additional research.

3146 Environmentally Relevant PFAS Exposures in a Zebrafish Model: Health Effects and Transcriptomic Assessment

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Per- and polyfluoroalkyl substances (PFAS) are mainly used as industrial or commercial surfactants and are present in many common household products, including nonstick cookware, popcorn bags, and cosmetics. PFAS consist of over 5,000 chemicals, all of which have a similar fully-fluorinated carbon chain that renders these chemicals extremely resistant to degradation, leading to environmental persistence and bioaccumulation. Despite phased out production in the United States, two of the most environmentally common PFAS compounds, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are still found in the ug/L range in blood samples from the general population. This range exceeds the EPA lifetime health advisory (HA) for PFOA, PFOS, or a mixture of these chemicals in drinking water, which is 70 ng/L (70 parts per trillion). Though most research to-date has focused on PFOA or PFOS alone, environmental exposures more typically include multiple chemicals at once. In fact, our lab detected PFOA and PFOS concurrently in the Lake Huron-to-Erie corridor at maximum levels of 4ng/L and 3ng/L, respectively. Previous research has linked high doses of PFAS to endocrine-related issues in adults, among other health concerns, but little is known about chronic, low level exposures during early development or to PFAS mixtures. Thus, we exposed embryonic zebrafish (*Danio rerio*) to environmentally relevant levels of PFOS, PFOA, and a 50:50 mixture of each compound at 0, 7, 70, and 700 ng/L from 4 to 120 hours post-fertilization. We then assessed developmental abnormalities, behavioral response to light/dark cycles to screen for neurobiological alterations, and genome-wide transcriptomic changes. We found significant behavioral changes that were chemical- and mixture-dependent, as well as altered gene expression in endocrine- and cancer-related pathways.

3147 Effects of Perfluoroalkyl Phoshinic Acid (PFPiA) on Behavior, Neurodevelopment, and DNA Methylation in Developing Zebrafish

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While recent studies have reported potential neurotoxicity and epigenetic alteration associated with exposure to several perfluoroalkyl substances (PFASs), the information is not only limited to only a few well-known compounds, but also their underlying mechanism are less understood. In the present study, we investigated the effects of 8:8 perfluoroalkyl phoshinic acids (8:8 PFPiA), one of the emerging PFASs but relatively under-studied, in early life zebrafish. We exposed different concentrations of 8:8 PFPiA (30, 100, 300, 1000, 3000, 10000 nM) to 4 hpf zebrafish embryos for 144 h. Although there were no significant changes in survival, hatchability, and malformation, the larvae exposed to PFPiA showed a decreased locomotor speed at 120 hpf comparing with controls. At 144 hpf, whole body samples were collected to examine the expression of genes involved in neurotoxicity. Genes related to thyroid hormone disruption and oxidative stress were also included considering its relationship with neurodevelopment and the findings from previous relevant studies. In addition, we estimated the alteration in global DNA methylation by ELISA method and specific gene methylation using bisulfite pyrosequencing assay, in order to understand the molecular mechanisms. The results of this study would enhance the understanding the adverse effects and molecular pathway of PFPiA and contribute to preventing risk of other similar PFASs.

3148 Gene Expression Changes in Maternal, Fetal, and Neonatal Tissues from Exposure to Hexafluoropropylene Oxide-Dimer Acid (HFPO-DA, GenX)

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The specific molecular mechanisms by which per- and polyfluorinated alkyl substances (PFAS) exert developmental toxicity in laboratory animals are not well described. Existing literature has focused on activation of peroxisome proliferator-activated receptor alpha (PPARα) and perturbation of the developing fetal tissues. Here, we utilized reverse transcriptase quantitative PCR (RT-qPCR) gene expression profiling arrays covering multiple signaling pathways to identify key genes and target tissues from pregnant Sprague-Dawley rats and offspring impacted by PFAS exposure as part of a set of broader experiments on the adverse effects of *in utero* PFAS exposure. Dams were dosed with hexafluoropropylene oxide-dimer acid (HFPO-DA; i.e., GenX) via oral gavage (water vehicle) from gestation days (GD) 14-18 (1-500 mg/kg/d) or GD 8 - postnatal day (PND) 2 (10-250 mg/kg/d) with maternal liver and fetal tissues (liver, heart, lung, kidney, thymus) collected on GD 18 and neonatal livers collected on PND 0. Maternal, fetal, and neonatal livers all displayed significantly altered expression of genes in the PPAR signaling pathway associated with all three PPAR isoforms - α, β/δ, γ. Each life-stage had distinct expression profiles; however, 7 genes (*Acadm, Acox1, Cpt1b, Ech1, Ehhadh, Fabp1*, and *Rxrg*) were significantly upregulated in maternal, fetal, and neonatal livers. Only neonatal livers displayed genes that were significantly downregulated (*Fabp2* and *Slc27a5*). Neonatal livers were also assessed for glucose metabolism signaling and 17 genes were significantly downregulated including *Ugp2*, *Aldob*, and *Agl*. Preliminary data on PPAR pathway gene expression changes in additional fetal tissues indicated that the overall rank order of tissues based on number of highly affected genes was: liver>thymus>heart>lung>kidney. The liver appears to be a key target tissue for toxic effects with multiple signaling pathways involved, not exclusively PPARα, and the specific genes affected are life-stage dependent. Ongoing research is investigating the maternal, fetal and neonatal liver expression of PPAR and glucose metabolism pathway genes from gestational exposure to a second understudied PFAS, Nafion byproduct 2 (NBP2), and a mixture of three PFAS (GenX, NBP2, and perfluorooctane sulfonate [PFOS]). *Abstract does not necessarily reflect US EPA policy.*

3149 Public Health Evaluation of PFAS Exposures and Breastfeeding: A Systematic Literature Review

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The Agency for Toxic Substances and Disease Registry (ATSDR) is working to address concerns from community members exposed to per- and polyfluoroalkyl substances (PFAS). Nursing mothers and health professionals have raised concerns about PFAS exposures through breastmilk. Fetuses and young children may be exposed to PFAS through trans-placental transfer and breastmilk, respectively. Longer breastfeeding duration is associated with greater levels of some PFAS in serum. ATSDR recommends that mothers who have been exposed to PFAS continue to breastfeed their infants; this is in accordance with guidance from American Academy of Pediatrics and the Centers for Disease Control and Prevention. The risks of exposure to environmental pollutants are unlikely to outweigh the well documented health benefits of breastfeeding for both mothers and infants. However, a formal review of evidence documenting any health effects associated with exposure to PFAS through breastmilk was not available. ATSDR is conducting a systematic review of observational human and experimental animal literature published through February 2018 to answer the following questions: (1) What is the evidence that exposure to PFAS through breastfeeding leads to a health outcome in humans or animals? and (2) Under what circumstances, if any, would a clinician recommend that a mother consider not breastfeeding her child? ATSDR followed the National Toxicology Program's Office of Health Assessment and Translation protocol to identify key questions using a PECO (population, exposure, comparator, outcome) framework and formulate criteria for inclusion and exclusion of published studies. ATSDR identified 4,297 unique records from 7 databases and included 37 articles for full-text review and some level of data extraction. The review included 8 animal studies in which the contribution of lactational exposure could be isolated from gestational exposure. This review will inform ATSDR's recommendations to nursing mothers using state-of-the-science evidence of the contribution of exposure to PFAS in breastmilk to health outcomes in infants and children. *The findings in this presentation have not been formally disseminated by the ATSDR and should not be construed to represent any agency determination or policy.*

3150 Elucidating Relationships between Internal Dose, Thyroid Hormones, and Hepatic Gene Expression Altered by Perfluoroalkyl Substances in Rats following 28-Day Oral Exposure

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Perfluoroalkyl substances (PFAS) are persistent in our environment and have been associated with numerous adverse health effects. The National Toxicology Program (NTP) recently evaluated the toxicity of seven perfluorinated chemicals to facilitate comparisons of toxicity across the chemical class and between sexes (NTP Toxicity Reports 96 and 97). Briefly, male and female Sprague-Dawley rats (N=10/exposure group/sex) were exposed to varying concentrations (5-1000 mg/kg/day) of either perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonate potassium salt (PFHSKslt), perfluorooctane sulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), or perfluorodecanoic acid (PFDA) for 28 days via oral gavage. Clinical pathology, thyroid hormones, hepatic gene expression, acyl-CoA oxidase activity, histopathology, plasma concentrations, and liver concentrations (males only) were evaluated at the end of the study. Key findings include dose-dependent decreases in thyroxine (T4) and triiodothyronine (T3), with minimal changes in thyroid stimulating hormone (TSH). Liver was a target organ, evidenced by increased liver weight, induction of hepatic *Acox1, Cyp4a1, Cyp2b1,* and *Cyp2b2*, histological lesions, and increased serum biomarkers of hepatobiliary injury. Using these data, we further investigated the relationship between these observations. In male animals, plasma and liver concentrations were strongly correlated (Pearson's $r \geq 0.88$) for all PFAS. Liver:plasma ratios ranged between 0.3 to 25 and tended to decrease with increasing dose, except for PFHxS where liver:plasma ratio increased with increasing dose. Hepatic gene expression was generally negatively correlated with T4 and T3, but not TSH, and more strongly in males than females. Significant correlations were seen more consistently among sulfonate PFAS. Thyroid hormones and liver endpoints were also examined in the context of all the measured endpoints to identify previously unidentified interactions. Overall, these analyses present efforts to better understand the health effects of PFAS, particularly the relationship between thyroid and hepatic effects.

3151 Perfluorinated Compounds Disrupt Wound-Healing Responses in Human Lung Fibroblasts (HFL-1)

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Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are persistent environmental contaminants that were used in fire retardants, food packaging, and textiles for decades. Despite phase-out of PFOS and PFOA, exposure from drinking water and dietary intake continues to be a health risk.

Detectable levels of PFOS and PFOA have been found in most people tested, raising concerns about their potential long-term health effects. Studies have demonstrated that PFOS and PFOA can accumulate in the lung and are associated with a higher risk of developing asthma, respiratory infection, and decreased lung function. However, the mechanisms underlying these potential health risks are unknown. We hypothesized that the harmful effects of PFOS and PFOA might be due to the dysfunction of normal wound healing responses by inhibiting fibroblast differentiation and inducing senescence. Human lung fibroblasts (HFL-1) were exposed to 50μM PFOS and PFOA alone or combined with 5ng/mL transforming growth factor-β (TGF-β) for 72h. Scratch assay was applied for measuring the wound healing response. Protein targets related to fibroblast-myofibroblast differentiation and senescence were determined as well. Using the scratch assay, wound closure was significantly inhibited by PFOS at both 48h (n=4, p<.001) and 72h (n=4, p<.05) compared to the TGF-β group. Furthermore, fibronectin and type-1 collagen induced by TGF-β were significantly reduced with PFOS (n=3, p<.001), but not PFOA. Moreover, there was no significant difference in α-smooth muscle actin (α-SMA). Interestingly, PFOS exposure resulted in increased p53, plasminogen activator inhibitor-1 (PAI-1), and p21, suggesting that PFOS may promote cellular senescence. Together, our data suggest that dysregulated wound healing processes might be a result of disturbing TGF-β signaling and promoting senescence by PFOS exposure. Future studies will examine PFOS modulation of TGF-β signaling and cellular senescence in order to understand how PFOS is negatively impacting lung health. *Supported by T32 ES007026.*

PS 3152 Developmental Toxicity of Perfluoroalkyl Substances Using Zebrafish

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Perfluoroalkyl substances (PFAS) are synesthetic compounds that are used in food packaging products, firefighting materials, electronics, cookware, carpets, furniture, clothing, and many other applications. PFAS are composed of a fluorinated carbon chain. PFAS are persistent in environment and bioaccumlative in organisms. The concerns of PFAS toxicity led to voluntarily phasing out of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by their manufacturer. PFOA and PFOS are both composed of 8 carbon chain (C8). Shorter chain chemicals (such as perfluorobutyrate (PFBA, C4) and perfluorobutane sulfonate (K-PFBS, C4)) and compounds with chemical modifications (such as GenX, C6) were used as a replacement to the long chain PFAS, (>C7 for COO^-- containing PFAS or >C6 for SO_3^-- containing PFAS), in order to increase their degradability potential. In this study, we compared toxicity of five PFAS in order to assess the role of chain length, functional group and chemical structure in their toxicity. We compared the toxicity of K-PFOS (CAS# 2795-39-3), PFOA (CAS# 335-67-1), K-PFBS (CAS# 2940-49-3), PFBA (CAS# 29420-49-3), and GenX (CAS# 13252-13-6) using Zebrafish (*Danio rerio*). To determine LC50 of each chemical, zebrafish embryos were exposed to a range of concentrations of each chemical within 1-hour post fertilization (hpf) for 120 hpf. The toxicity of these compounds was assessed by monitoring the survivability every 24 hours through 120 hpf. 120hpf-LC50 were determined using SPSS-23 software. In addition, behavioral analysis using a visual motor response test was performed. For behavioral analysis, we used concentrations of 0, 4, 40, 400, and 4000 part per billion (ppb). The exposure was terminated at 72hpf and the test was done at 120hpf. Results showed that the 120hpf-LC50s are 24.04 part per million (ppm) for PFOS, 451.15 ppm for PFOA, 2578.08 ppm for PFBS, >10000 ppm for PFBA, and >7000 ppm for GenX. Toxicity ranking is K-PFOS> PFOA> K-PFBS >PFBA, GenX. Based on these results, we can conclude that toxicity increases with increasing the chain length. Also, presence of sulfonate group increased toxicity for PFAS of a given chain length. Behavioral analysis showed that embryonic exposure to K-PFOS, PFOA or GenX induced hyperactivity in larvae, while PFBA and K-PFBS caused hypoactivity. Future work is identifying the mechanism behind the observed behavioral changes.

PS 3153 A Comparison of Effects and Modes of Action of Perfluorooctanoic Acid (PFOA) and Hexafluoropropylene Oxide Dimer Acid (HFPO-DA, or GenX) in Liver and Placenta from CD-1 Mice Exposed during Pregnancy

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Perfluorooctanoic acid (PFOA) is a perfluoroalkyl substance (PFAS) associated with adverse pregnancy outcomes in mice and humans, but little is known regarding one of its replacements, hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). GenX was selected to replace PFOA due in part to its quicker elimination in both human and rodents but has received public scrutiny in North Carolina after reports of high levels of GenX contamination in the Cape Fear River. We compared the toxicity of PFOA and GenX in pregnant mice and their developing embryo-placenta units. Pregnant CD-1 mice were exposed daily to PFOA (0, 1 or 5 mg/kg) or GenX (0, 2 or 10 mg/kg) via oral gavage from embryonic day (E) 1.5 to 11.5 or 17.5. Maternal clinical chemistry, maternal liver and placental histopathology, embryo and placental weight, internal dosimetry, and placental thyroid hormones were measured. Accumulation of GenX in maternal serum, amniotic fluid, and whole embryos was comparable to levels in mice exposed to PFOA, but GenX accumulated to a much lesser extent than PFOA in the maternal liver (5 mg/kg PFOA: 159.2 ± 22 μg/g; 10 mg/kg GenX 14.2 ± 8 μg/g). GenX exposure recapitulated many known effects of PFOA on the liver/metabolic system, including significantly increased gestational weight gain, reduced serum triglycerides and 100% incidence of hepatocellular cytoplasmic alteration. Subcellular changes induced by PFOA and GenX in hepatocytes included reduced glycogen, increased mitochondria and peroxisomes, and altered rough endoplasmic reticulum. Incidence of placental lesions was similar for both 5 mg/kg PFOA (68%) and 10 mg/kg GenX (83%), but lesion subtype incidences were compound-specific. Placenta labyrinth atrophy was more common in GenX-exposed placentas (10 mg/kg GenX: 46%; 5 mg/kg PFOA: 7%) and labyrinth congestion was more common in PFOA-exposed placentas (5 mg/kg PFOA: 57.5%; 10 mg/kg GenX: 23%). Placental thyroxine was disrupted in mice exposed to 10 mg/kg GenX, but not altered in mice exposed to PFOA. Taken together, these data suggest that PFOA and GenX likely utilize divergent mechanisms of toxicity in the placenta. Transcriptomic analyses of maternal livers, placentas, and embryo livers are in progress to characterize the shared and divergent molecular pathways used by PFOA and GenX to exert toxic effects in the maternal-embryo-placental unit.

PS 3154 Development of an AOP Network for the Developmental Effects of Exposure to the PFAS

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There is growing global concern about the health effects of PFAS due to widespread exposure from a variety of sources. We are developing an AOP network for PFAS from the literature and from our own developmental studies with rats. The goal is to link *in vitro* Molecular Initiating Events (MIEs) and *in vivo* Key Events (KEs) to adverse fetal and postnatal effects of individual PFAS and mixtures of PFAS. *In vitro* data demonstrate that the PFAS (HFPO-DA, PFNA, PFOS, PFOA, PFBS, PFMOAA and nafion byproduct 2 (NBP2)) and fatty acids (FA) (oleic, linoleic, octanoic) are PPAR α and γ agonists but 6:2 FTOH is not. Only FA activated PPAR δ. None of the PFAS or FA are AR or GR agonists, whereas 6:2 FTOH appears to be a weak ER agonist. In order to identify KEs related to PFAS developmental toxicity, we dosed pregnant rats from gestational day (GD) 14 to 18, we found that HFPO-DA (GenX) altered fetal and maternal liver mRNA expression for genes in the PPAR and lipid pathways, increased maternal but not fetal liver weight, altered maternal serum lipid profiles, and reduced maternal serum T3 and T4. We also are examining the effects of PFAS on gene pathways in other fetal tissues including the thymus, heart, lung, brain and placenta. Although fetal viability and body and liver weight were unaffected by GD 14 to 18 exposure, in a pre-postnatal study dosed from GD 8 to postnatal day 2, HFPO-DA reduced pup weight and viability at dose levels that did not induce overt maternal toxicity. HFPO-DA also increased liver PPAR gene expression and liver weight in the dam and pups and reduced neonatal liver glycogen storage, which by itself could result in neonatal mortality. Similar studies on the effects of NBP2 and a PFAS mixture (HFPO-DA, NBP2 and PFOS) are ongoing. To date, we have found that NBP2 and the mixture of PFAS (dose additive) also reduced neonatal survival and growth. Taken together, these data indicate that PFAS act via multiple MIEs, disrupting multiple KEs that result in diverse effects in fetal, neonatal and maternal tissues. MIEs include, but may not be not limited to, PPAR α and γ and ER agonism. The *in vitro* PPAR agonism EC_{50}s did not correlate well with the reduced neonatal viability following *in utero* exposure and PFAS-induced hepatomegaly and induction of the liver PPAR pathways also were poorly correlated with neonatal mortality. In summary, tissue- and life-stage-specific AOP networks that account for multiple MIEs likely will be needed to accurately describe the developmental effects of *in utero* PFAS exposure. *This abstract does not reflect Agency policy.*

3155 Impact of Environmentally Relevant Concentrations of PFAS on Global Methylation Patterns in a Placental *In Vitro* Model

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Per- and polyfluorinated compounds (PFAS) are a class of fluorinated compounds human populations are routinely exposed to. Common routes of exposure include drinking water, non-stick pans, carpets and other surfaces treated with PFAS to repel water. The ATSDR has acknowledged that exposure to PFAS, including perfluorooctanesulfonic acid (PFOS) and perfluoro-n-octanoic acid (PFOA), is linked to adverse developmental outcomes such as preeclampsia and low infant birth weight. Recently, a newly developed PFAS, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, also known by the trade name GenX, has been found in abnormally high concentrations in drinking water sourced downstream of an industrial production facility in the Cape Fear river basin in North Carolina. In light of ongoing exposure and the established link between PFAS and adverse developmental outcomes, it is essential to determine the developmental impact of newly developed PFAS such as GenX. Since epigenetic changes play a crucial role in regulating cellular processes during development, it is important to understand how PFAS impact epigenetic regulatory mechanisms. To answer this question, we analyzed whether exposure to varying concentrations of PFAS affect genome-wide 5mC methylation patterns in JEG3, an *in vitro* trophoblast model. Cells were treated using 100 ng/ml and 1000 ng/ml of PFOS, PFOA and GenX in serum and serum free media for 24 hours; genomic DNA was harvested; and an Enzyme-Linked Immunosorbent Assay (ELISA) was used to analyze global LINE-1 DNA methylation. While no significant differences from the control were observed at $p<0.05$, dose-dependent trends were observed. Specifically, increasing PFOS levels led to decreasing global methylation. Additionally, PFOA exhibited the opposite trend, with increasing PFOA levels leading to increasing global methylation. Further research is needed to elucidate the specific impact of PFOS, PFOA and GenX on global methylation patterns and on methylation patterns of specific genes and regulatory elements.

3156 Development of a Gas Chromatography-Mass Spectrometry Method for Simultaneous Determination of Various Per- and Polyfluoroalkyl Substances (PFAS) in Biological Media

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Per- and polyfluoroalkyl substances (PFAS) are a large yet diverse class of compounds that are widely used for their stain-resistant, waterproofing, and friction-reducing properties. In general, PFAS compounds are persistent in the environment and bioaccumulate in wildlife and humans. Exposure occurs primarily through ingestion of drinking water but can also occur through inhalation and skin absorption. Despite widespread exposure, the toxicity data for PFAS are limited, particularly for some of the shorter and branched chain compounds that are increasing in production and use. The objective of this work was to develop a method to simultaneously quantitate various PFAS in support of studies investigating the clearance of PFAS in hepatocytes. Sixteen PFAS were selected, including fluorotelomer alcohols, polyfluorinated alcohols, a fluorotelomer-based methacrylate, a polyfluoroalkyl ether, a polyfluoroalkyl ether alcohol, a perfluoroalkyltrifluoromethane-sulfonate, and an *N*-alkylperfluoroalkane sulfonamidoethanol. The analytes were 4:2 fluorotelomer alcohol, 6:2 fluorotelomer alcohol, 8:2 fluorotelomer alcohol, 4:4 fluorotelomer alcohol, 11:1 fluorotelomer alcohol, heptafluorobutanol, 1H,1H,5H-perfluoropentanol, 3-(perfluoropropyl)propanol, dodecafluoroheptanol, 1-pentafluoroethylethanol, hexafluoroamylene glycol, 6:2 fluorotelomer methacrylate, tris[(trifluoroethoxy)methane], 1H,1H,8H,8H-perfluoro-3,6-dioxaoctane-1,8-diol, 2,2-difluoroethyl triflate, and *N*-ethyl-N-(2-hydroxyethyl)perfluorooctane sulfonamide. Media standards (0.1 to 10 µM) were prepared by adding 10 µL solution containing the analyte mixture to 100 µL of hepatocyte incubation media containing Williams E media with 15 mM HEPES and extracting with 90 µL of ethyl acetate. Following centrifugation, the organic layer was analyzed by gas chromatography-mass spectrometry, using single ion monitoring. A DB-WAX column was used with oven temperature ramped from 40°C to 230°C in 19 min. Different columns and instrument conditions were evaluated to optimize chromatography and ionization, and the extraction process was optimized using different solvents, ratios, and degree of centrifugation. Multi-point calibration curves were assessed in solvent and media to evaluate sensitivity, linearity, recovery, and matrix effects. Media standard curves were linear ($r \geq 0.99$) over the range 0.1 to 10 µM for all analytes. Mean recoveries were 93-115%, and matrix effects were minimal. These data demonstrate that the method is suitable for the analysis of 16 PFAS in hepatocyte suspension, in support of *in vitro* screening studies.

3157 Perfluoroalkyl Carboxylic Acids Are Substrates of the Bile Acid Transporter NTCP

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Sodium/taurocholate cotransporting polypeptide (NTCP) is a sodium-dependent bile acid transporter located at the basolateral membrane of hepatocytes, important for the enterohepatic circulation of bile acids. We previously had demonstrated that NTCP can transport several perfluoroalkyl sulfonates. In addition, our previous results demonstrated that organic anion transporting polypeptides (OATPs) 1B1, 1B3 and 2B1 can transport perfluoroalkyl carboxylates with 8 and 9 carbon atoms. In the current study we determined to what extent the perfluoroalkyl carboxylates perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), or perfluorodecanoic acid (PFDA) are substrates of NTCP. Human embryonic kidney cells (HEK293) stably expressing NTCP were plated on poly-D-lysine-coated 24 well plates, and uptake of sodium-dependent ^{3}H-taurocholate was measured as a positive control. Time- and concentration-dependent uptake of PFOA, PFNA, and PFDA was quantitated by LC-MS/MS. Protein concentrations were determined by BCA assay. PFOA, PFNA, and PFDA inhibit NTCP. In addition, all compounds are transported by NTCP. Uptake is time- and concentration dependent. Kinetic experiments suggest that PFOA, PFNA and PFDA are transported with relatively low affinities. Similar to perfluoroalkyl sulfonates, PFOA, PFNA, and PFDA can inhibit NTCP-mediated taurocholate uptake and all three compounds are also substrates of NTCP. In addition to our previous data that demonstrated liver-expressed OATP1B1, 1B3 and 2B1 can transport PFOA and PFNA, our current study provided additional evidence that NTCP is also important for the enterohepatic circulation of PFOA, PFNA, and PFDA.

3158 Transcriptomic Responses in Livers of GenX-Treated Mice Demonstrate Upregulation of PPAR Signaling and Related Pathways

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GenX was developed as an alternative to long-chain per- and poly-fluoroalkyl substances (PFAS). It has been postulated that the underlying mechanism of GenX-induced liver lesions in mice involves PPARα activation. To investigate alterations in PPARα signaling at the mRNA level, as well as other potential molecular signals related to GenX-induced liver effects, transcriptomic analysis was conducted in liver sections from male and female mice previously exposed to up to 5 mg/kg bw GenX by oral gavage for 90 days (2010). The RNA sequencing platform TempO-Seq, which is amenable to FFPE tissue sections, was used to identify differentially expressed genes for each treatment group. Pathway enrichment analysis was conducted using gene sets that represent biological states and known canonical pathways. There was no significant gene set enrichment at 0.1 mg/kg, while significant enrichment of PPAR signaling was prominent in both sexes at 0.5 and 5 mg/kg. PPAR signaling and fatty acid metabolism were among the most significantly enriched genesets, and two genesets specific to the PPARα subtype were significantly enriched. Benchmark dose modeling was conducted, and PPAR signaling pathways were again found to be significantly enriched among dose-responsive genes, as were mitotic cell cycle gene sets. Additionally, down-regulation of coagulation cascade proteins was observed in both sexes at 0.5 and 5 mg/kg, which has been reported to occur during hyperplasia-mediated liver proliferation in mice. The original histopathological analysis of these liver sections (2010) reported increased 'hepatocyte single cell necrosis' in the liver at 5 mg/kg; however, updated diagnostic criteria recommend that 'single cell necrosis' be diagnosed as either necrotic or apoptotic cell death. A reevaluation of liver tissue from the same FFPE tissues demonstrated that liver lesions previously diagnosed as 'single cell necrosis' were in fact apoptosis, a finding that was corroborated by immunostaining for activated caspase-3. GenX also increased mitosis in the liver at the same doses as apoptosis, which is consistent with rodent-specific cell cycle changes induced by PPARα activators. These results indicate that the liver changes observed in GenX-treated mice occur via a mode of action (MOA) involving PPARα, a finding important for risk assessment as this MOA has been determined to not be relevant to humans.

3159 Development of a Maximum Allowable Dose Level for Perfluorooctanoic Acid and Comparison of Exposures from Consumer Products, Drinking Water, and Natural Food Sources

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Perfluorooctanoic acid (PFOA), a polyfluoroalkyl substance, which is used in the production of fluoropolymers, is an environmentally persistent industrial contaminant. The US EPA concluded that PFOA may be developmentally toxic to humans based on toxicology studies, and, thus, PFOA was added to the California Proposition 65 (Prop 65) list by the authoritative bodies mechanism. As there is no safe harbor level (SHL or Maximum Allowable Dose Level, MADL) for PFOA, the current work was undertaken to: derive a MADL; determine if PFOA exposure from consumer products, drinking water, or natural food sources would be likely to exceed the MADL; and, determine which sources of exposure would be of greatest concern. Prop 65 requires businesses to provide a clear and reasonable warning if they sell products or discharge effluent into drinking water sources that would result in exposures above a SHL. From the most sensitive PFOA developmental study, a Lowest-Observed-Effect Level (LOEL) of 0.3 mg/kg/d was identified based on findings of increased femoral periosteal area and decreased mineral density in tibias. A MADL of 1.7 mcg/d was calculated from the LOEL [(0.3 mg/kg/d/10)/1000 x 58 kg x 1000 mcg/mg]. Data from the Unregulated Contaminant Monitoring Rule were used to determine average (0.028 mcg/L) and maximum (0.053 mcg/L) concentrations of PFOA in CA drinking water which corresponded to exposures of 0.056 and 0.106 mcg/day (assuming 2 L/d), respectively. Fruits, vegetables, and flour substantially contributed to PFOA intake from the diet and median and high-level intakes from these sources were determined to be 0.2 and 0.5 ng/kg bw/d, respectively, or 0.0116 and 0.029 mcg/day. A worst-case exposure scenario to PFOA from consumer products was determined for standard 10″ pans coated with polytetrafluorethylene (PTFE) resulting in both oral exposure from eating the cooked food and dermal contact from handling and cleaning the pan. The sum of oral and dermal exposure to PFOA from use of the PTFE-coated pan was determined to be 0.042 mcg/day. Based on this work, exposures to PFOA from cooking pans (a worst-case scenario), drinking water, or natural food sources would not be expected to exceed the calculated MADL for PFOA. Exposure to PFOA from drinking water sources resulted in the highest level of exposure which may be a target for future efforts to reduce PFOA exposures to average Californians.

3160 Evaluation of Perfluorooctanoic Acid (PFOA) Replacement Chemicals on Pancreatic Toxicity

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Per- and polyfluoroalkyl substances (PFAS) are used in a variety of industrial applications including production of surface repellant coatings, fire-fighting foams, and fluoropolymer synthesis. Due to their environmental persistence, bioaccumulation, and toxicity of the legacy PFAS, such as perfluorooctanoic acid (PFOA), other perfluoroether carboxylic acids (PFECAs) have been introduced to replace PFOA as a processing aid in fluoropolymer production. Previously, we demonstrated that PFOA triggered ductal hyperplasia, oxidative stress and inflammation in the mouse pancreas after a 7 day exposure. In addition, we have shown that PFOA induces endoplasmic reticulum (ER) stress, triggering the unfolded protein response (UPR) in the mouse pancreatic acinar cell line 266-6 and *in vivo* in the mouse pancreas. While the toxicity profiles of the legacy PFAS have been studied, little is known of the toxicities caused by exposure to the alternative products, for which environmental and biological contamination has been documented globally. In comparison to PFOA, we evaluated the effects of two PFECA compounds on pancreatic toxicity *in vitro* and *in vivo*: ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX) and perfluoro(2,5-dimethyl-3,6-dioxanonanoic acid) (HFPO-TA). In the 266-6 cell line, GenX exhibited less effects on cell viability as compared to PFOA, while HFPO-TA caused a greater cytotoxicity. This same rank order was observed when stimulation of the UPR was monitored by ATF4 and CHOP mRNA induction. *In vivo*, mice were exposed to 5 and 10 ppm GenX, 0.5 and 1.5 ppm HFPO-TA, and 5 ppm PFOA in drinking water for 7 days. To measure the effects on the pancreas, serum lipase and amylase were measured. While no changes were observed in lipase levels, exposure to 10 ppm GenX increased amylase, while 1.5 ppm HFPO-TA exposure decreased amylase levels. Examination of mRNA levels demonstrated that all three PFAS altered markers of ER and oxidative stress in the pancreas, with HFPO-TA exhibiting the strongest effect. As pancreatic damage, ER and oxidative stress have been linked to chronic diseases such as diabetes and pancreatic cancer, our findings suggest that further toxicological evaluation of the replacement PFAS with respect to pancreatic toxicity are warranted.

3161 The Interaction with and Transport of Three Perfluoroalkyl Sulfonates by Renal Organic Anion Transporters

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are persistent environmental chemicals and some have long serum elimination half-lives in humans. There is evidence suggesting that some PFAS such as perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) are actively reabsorbed by the tubules of the kidney which could be a contributing factor to their long half-lives. The aim of this study is to determine the role of several renal organic anion transporters (OAT1, OAT3, OAT4, URAT1, and OATP1A2) in the reabsorption of selected PFAS by the kidney. We undertook this study to measure the inhibitory effects of perfluorobutane sulfonate (PFBS), PFHxS, and PFOS against the activity of these transporters, which were transiently expressed in HEK293 cells, using radiolabeled probe substrates. We also quantitatively measured the time-dependent uptake of PFBS, PFHxS, and PFOS using LC-MS/MS. Our preliminary data suggested that, for the most part, PFBS and PFHxS inhibited the transport activity of these selected transporters to a greater extent than PFOS, with the greatest inhibition observed in the transport activity of OAT3 and OAT4. In addition, we also observed that: 1) OAT1 and OAT4 transported PFBS, PFHxS and PFOS; 2) OAT3 and URAT1 transported PFBS and PFHxS; and 3) OATP1A2 transported PFOS exclusively. These results demonstrate that the renal organic anion transporters interact with and transport perfluoroalkyl sulfonates and is consistent with these transporters having a role in the reabsorption of these compounds by the kidney.

3162 iPSC-Derived Hepatocytes and Transcriptomics Reveal Mechanisms of PFOA- and PFOS-Induced Developmental Hepatotoxicity

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Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are persistent organic pollutants with myriad of toxic effects including developmental toxicity and hepatotoxicity. Our studies have shown that PFOA and PFOS degrade HNF4α, the main hepatic differentiation factor, resulting in significant hepatotoxicity. Given that HNF4α is critical for liver development and the fact that PFOA and PFOS can cross placenta, we hypothesized that PFOA and PFOS affect embryonic liver development. We investigated the effects of PFOA and PFOS on embryonic liver development using human iPSC-derived hepatocyte model, which mimics all stages of human liver development. iPSC cells were exposed to PFOA and PFOA at 10 μM concentration starting from either definitive endoderm stage, hepatic endoderm stage or hepatotoblast stage till mature hepatocyte stage. Transcriptomic changes were determined using RNA sequencing (RNAseq) and IPA analysis. PFOA induced significantly more gene expression changes than PFOS and affected all three stages of liver development. PFOA inhibited genes involved in thyroid hormone synthesis, cAMP, glutamate receptor, and integrin signaling; and activated gene involved in lipid, sterols, cholesterol transport, NRF2 pathway, and ROS-mediated signaling over the differentiation period. Target genes of HNF4α and several related transcription factors including HNF1α and CEBP/β were suppressed whereas targets of NR5A1, FOXA3, SIRT1, and CBX5 were induced by PFOA treatment during hepatic differentiation. The major effect of PFOS was to inhibit several pathways during hepatoblast to hepatocyte maturation period. PFOS inhibited genes in cAMP, eNOS, Gas, FGF, calcium, glutamate, and Apelin signaling pathways; and induced genes in melatonin and nicotine degradation pathways and citrulline biosynthesis. Interestingly, PFOS inhibited genes in cancer and cell movement, and induced genes in glucuronidation of sterols. PFOS inhibited target genes of VEGF, GLI, FGF2, CREB, NF-κB, SP1, and several other transcription factors involved in hepatocyte maturation while inducing expression of PXR and NUPR1 targets. Taken together, these data indicate PFOA and PFOS have significant and specific effects on embryonic liver development, which should be considered in risk assessment.

3163 Low-Dose PFOS Exposure Alters the Placental Transcriptome in C57BL/6 Mice

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The placenta plays a vital role in fetal development and health. As the primary conduit for fetal development, the placenta mediates nutrient transfer, waste elimination, gas exchange, hormone production, and development of the fetal immune system. Per- and polyfluoroalkyl substances (PFAS) are a class of man-made, ubiquitous environmental toxicants known to cause adverse health effects. Perfluorooctanesulfonic acid (PFOS), the most prevalent PFAS member, consists of a synthetic perfluorinated eight-carbon backbone with a sulfonic acid head group. Evidence suggests that PFOS can induce developmental toxicity in humans and rodents following placental transfer and has been associated with low birth weight in humans. Furthermore, PFOS is detected in both human and rodent umbilical cord blood and in breast milk. The overarching hypothesis of the work is that PFOS exposure impacts placental health and function through modulation of the placental transcriptome. The aim of this study was to elucidate the effects of developmental PFOS exposure at 3 ppm (~0.3 mg/kg/day) or 30 ppm (~3 mg/kg/day) per day in feed on the placental response *in vivo* using 10-week old C57BL/6 timed-pregnant dams. On gestational day 1 (GD1), dams were assigned to one of the following blinded experimental diets and fed *ad libitum*: 1) Standard chow diet, 2) 3 ppm PFOS (w/w) or 3) 30 ppm PFOS (w/w). Dams were euthanized at GD17 and placentae collected. Fetal weight collection confirmed statistical significance in low birth weight association to PFOS exposure with a more robust weight reduction in the 3 ppm PFOS-treated. RNA integrity was confirmed, libraries were prepared, and next-generation sequencing (RNA-Seq) was performed to investigate the impact of PFOS exposure on the placental transcriptome. Our studies indicate upregulation of Phospholipase C signaling, Actin Cytoskeleton Signaling, Rho Family GTPase, EIF2 Signaling, Oxidative Phosphorylation signaling pathways, with 3 ppm PFOS inducing a more robust transcriptional response. In addition, at 3 ppm PFOS elicited a fold change of ~2.0 in the Gzmf, Emilin1, Gzmg, Slit1, and Lrp1 genes. Interestingly, gene Hsd3b1 resulted in a fold change of ~2.0 within the PFOS-treated. Hsd3b1 is responsible for the conversion of pregnenolone to progesterone in the placenta. In summary, our data suggest that PFOS, at relatively low concentrations in diet, can induce a significant placental response.

3164 Exposure of Perfluorooctanesulfonic Acid (PFOS) as a Potential Risk Factor for Late-Onset Alzheimer's Disease (LOAD)

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Poly- and perfluoroalkyl substances (PFAS) are a class of emerging persistent organic pollutants (POPs) linked to neurotoxicity and are considered major risks to the ecosystem and human health. Of particular concern is the legacy PFAS perfluorooctanesulfonic acid (PFOS), which has been widely used in industrial, commercial and household applications due to its unique physicochemical and amphipathic properties. PFOS is very stable in the environment as it is resistant to biodegradation, direct photolysis, hydrolysis and photo-oxidation. Furthermore, PFOS cannot be metabolized and is not well excreted, leading to an elimination half-life in humans at about 5.4 years. Even though levels of PFOS exposure have decreased over the last decade, its toxicity is attracting a lot of attention, particularly because of the ability of PFOS to cross the brain blood barrier, placenta and into breast milk. Studies suggest that early life exposure to environmental toxicants influences the pathogenesis of neurodegenerative diseases later in life. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accounts for 60-70% of dementia cases worldwide according to the World Health Organization. Two physiological features of AD are extracellular senile plaques consisting of β-amyloid (Aβ) peptide that is cleaved from a larger protein called the amyloid precursor protein (APP), and intracellular neurofibrillary tangles (NFTs) containing hyperphosphorylated tau protein. This hyperphosphorylation of tau protein is regulated by activated kinases such as glycogen synthase kinase-3 beta (GSK3β). Thus, the aim of this study is to investigate the association between PFOS exposure and AD-related biomarkers. Differentiated SH-SY5Y neuroblastoma cells were exposed to PFOS concentrations of 0-100 μM for 24, 48, and 72 h. After 48 h, PFOS treatment at 50 μM resulted in an increase of GSK3β protein expression and phosphorylated tau (P-tau) at ser 404 by 1.29- and 1.69-fold vs control respectively. Data presented herein suggests that PFOS exposure may perturb AD-related biomarkers such as tau, P-tau, and GSK3β, indicating that PFOS is a potential risk factor for Alzheimer's disease. *In vivo* studies are underway to validate these findings.

3165 Perfluorooctanoic Acid Alters the Function and Global Proteome of Differentiating Primary Human Villous Trophoblasts

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Perfluorooctanoic acid (PFOA) is a widely used industrial surfactant and a major contaminant of developmental concern due to its environmental persistence. In humans and rodents, maternal PFOA exposure has been linked to adverse pregnancy outcomes, however the specific cellular and molecular mechanisms are unclear. The human placenta is the interface between the maternal and fetal compartments, and is a potential target of various environmental stressors including PFOA. Placental cytotrophoblasts (CTBs) play critical roles in implantation, uterine infiltration, and vasculature development, and perturbations in CTB function underlie common pregnancy complications (*e.g.,* preeclampsia, preterm birth). *In vitro*, primary human villous CTBs differentiate towards an invasive phenotype, correlating with their *in vivo* characteristics. We previously demonstrated the utility of this model system (on functional and transcriptomic levels) to evaluate the toxicity of persistent pollutants such as brominated flame retardants. To test the hypothesis that CTBs are sensitive to PFOA exposure, we assessed primary human CTB differentiation using functional and proteomic approaches. Second trimester CTBs were treated with vehicle (0.1% DMSO) or PFOA (25 μM, 100 μM) for 39 hr and invasion was assessed using the Transwell assay. PFOA significantly increased the invasive ability of CTBs at concentrations independent of cytotoxicity. To identify the molecules driving these observed changes, protein homogenates of CTBs treated with vehicle or PFOA (100 μM) were collected during differentiation (24 hr). The global proteome was profiled using sequential window acquisition of all theoretical mass spectra (SWATH-MS). Signaling molecules associated with invasive and inflammatory processes were differentially expressed after PFOA exposure, including ITGA6/B4 (integrin alpha 6/beta4), COX2 (cyclooxygenase-II), MIF (macrophage migration inhibitory factor), and GPX7 (glutathione peroxidase 7). Our results suggest that PFOA alters key proteins involved in CTB differentiation and functionality *in vitro*, via induction of cellular invasion.

3166 Toxicokinetic Processes Differ between Perfluorinated Alkyl Acid Carboxylates and Sulfonates in Zebrafish Embryo

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Perfluorinated alkyl acid carboxylates and sulfonates (PFAA) are a group of extremely persistent organofluorine chemicals. PFAAs have been found to accumulate in wildlife and human globally. To date, understanding is still limited how the various PFAA structures (e.g. alkyl chain length, functional group) impact the toxicokinetics (TK) and, consequently, the toxicity. We studied the TK processes of perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and perfluorobutanesulfonic acid (PFBA) using the zebrafish (*Danio rerio*) embryo (ZFE) model. To this end, we exposed ZFE from two hours post fertilization (hpf) until 120 hpf to each of the four PFAAs at two or three different concentrations. We measured exposure concentrations in the medium and internal concentrations in the whole ZFE at nine time points using UPLC-MS/MS. Additionally, we sampled 120 hpf old ZFE exposed to PFOS, PFOA and PFHxS to visualize distributions in ZFE. ZFE were embedded in a mixture of 5% carboxymethyl cellulose (w/w) and 10% gelatin (w/w). Embedded tissues were frozen, cut in 18 μm thick slides and mounted on glass slides. The lateral distribution of PFAA was determined using MALDI-MS imaging. Determined bioconcentration factors differed four orders of magnitude with PFOS (4000 L/kg) > PFHxS (200 L/kg) > PFOA (50 L/kg) > PFBA (0.8 L/kg). The apparent toxic potency varied about three orders of magnitude between these four PFAAs when based on effective external concentrations. However, after correction for bioconcentration, the effective internal concentrations became similar, reducing the differences in toxic potency to only three-fold difference. Moreover, PFOS and PFHxS tended to accumulate in the ZFE brain, while PFOA tended to accumulate in the vascular system. These differences in distribution may explain reported organ-specific toxicity of PFAAs. In summary, our data indicate that both alkyl chain length and the functional group of PFAAs influence the TK processes, which in turn directs toxic potency and target organ toxicity.

3167 Interspecies Extrapolation of Toxicity Data for Perfluorohexanoic Acid (PFHxA)

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It is the practice of the US EPA to use a margin of exposure (MOE) approach in its human health risk characterization of new chemicals under the Toxic Substances Control Act. EPA compares the MOE of the new chemical to a benchmark (acceptable) MOE derived by applying a combined uncertainty factors (UF) based on (1) interspecies extrapolation (UFA); (2) intraspecies extrapolation (UFH); and (3) LOAEL-to-NOAEL extrapolation (UFL). Default UFs of 10 are commonly applied such that the benchmark MOE for a new chemical is typically 100 or 1,000. The interspecies UF can be subdivided into pharmacokinetic (PK) and pharmacodynamic (PD) components, and each component is customarily assigned a value of 3. However, if chemical- and species-specific data are available, the PK component of the interspecies UF can be replaced with a data-derived extrapolation factor (DDEF). In 2015, the EPA Risk Assessment Division developed a DDEF for interspecies extrapolation of toxicity data for perfluorohexanoic acid (PFHxA) of 768 and a subsequent benchmark MOE of 23,000. The derived DDEF was based upon the ratio of volumes of distribution between humans and rats (estimated to be 1), and the ratio of serum elimination half-lives for humans ($t_{1/2}$ = 768 hours) and male rats ($t_{1/2}$ = 1.0 hours). However, toxicokinetic data for PFHxA demonstrate that serum elimination half-lives for PFHxA are proportional to body weight across mice, rats, monkeys, and humans. This finding supports the use of allometric body weight scaling to the ¾ power for the PK component of the interspecies UF, consistent with recent assessments of PFHxA by other authoritative bodies. Use of allometric body weight scaling supports a much lower benchmark MOE, closer to 100, for PFHxA. These results highlight how different interspecies extrapolation methods can lead to disparate benchmark MOEs, which could ultimately lead to different decisions regarding the need for risk management.

3168 PFOA, PFOS, and PFNA Affect Cholesterol Metabolism in Human HepaRG Liver Cells

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Per- and polyfluoroalkyl substances (PFASs) are omnipresent, very persistent in the environment and present in the food chain. Epidemiological studies have shown an association between serum levels of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and increased total and LDL serum cholesterol levels. These human data have been used by the EFSA to derive a tolerable weekly intake for these chemicals. However, causality has been questioned, amongst others given that animal studies, as well as a human phase 1 dose-escalation trial (both at high doses), show a PFAS-induced decrease of serum cholesterol levels. Also, although PFASs have been shown to act as PPAR agonists, the role of PPAR activation in PFAS-induced changes in cholesterol metabolism is not known. Studies with human cell models can provide more insight in the mode of action underlying PFAS-induced effects on cholesterol metabolism, enabling better interpretation of the human epidemiological data. The present study assessed the effects of PFOA, PFOS and perfluorononanoic acid (PFNA) on whole genome gene expression in human HepaRG liver cells using DNA microarray analysis. Gene set enrichment analysis (GSEA) of the microarray data pointed to several cellular processes affected upon PFAS treatment. Gene sets related to cholesterol biosynthesis were downregulated for PFOA, PFOS and PFNA. Gene sets related to PPAR signalling were only enriched upon PFOA treatment, but the expression of a number of PPAR responsive genes was also significantly induced by PFOS and PFNA. Concentration-response analyses for selected genes related to cholesterol biosynthesis and PPAR signalling indicated that PFNA appeared to be the most potent inhibitor of cholesterol biosynthesis, whereas PFOA appeared to be the most potent PPAR activator, suggesting that PFOA-, PFOS- and PFNA-induced decrease of cholesterol biosynthesis is not directly related to PPAR activation. Besides effects on cholesterol biosynthesis and PPAR signalling, the present study also points to other gene sets in common for PFOA, PFOS and PFNA, which are related to PERK/ATF4 signalling (upregulated), tRNA aminoacylation (upregulated), amino acid transport across the plasma membrane (upregulated), and glycolysis/gluconeogenesis (downregulated). These data indicate possible other cellular processes that may also play a role in PFAS-induced adverse effects.

3169 *In Vitro* Hepatic Clearance of Per- and Polyfluoroalkyl Substances (PFAS)

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Per- and polyfluoroalkyl substances (PFAS) are man-made chemicals that have been used in a variety of industries throughout the world. The two most studied PFAS compounds are perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) which have both been shown to be persistent in both the environment and in humans as neither is broken down in the environment or metabolized in humans. To better understand the significance to public health a two sets of 75 PFAS compounds was selected to cover different classes of PFAS. The goal of this work was to determine whether or not these compounds are hepatically cleared via metabolism using primary human hepatocytes (PHHs). Allowing for the potential assessment of the persistence of these chemicals in humans. In total, 27 of the 75 PFAS in set 1 were amenable to liquid chromatography mass spectrometry (LCMS) using ESI. These 27 PFAS compounds were subjected to an initial screen for hepatic clearance where 10-donor pooled primary human hepatocytes (PHHs) were incubated with 10 µM of PFAS for 0 and 4 hours at 37 C. Heat inactivated PHHs were also incubated with 10 µM of PFAS for 0 and 4 hours at 37 C as a control. Comparison of peak area ratios (4 hours/0 hours) for the active and heat inactivated PHHs was completed and a t-test was used to determine whether or not there was a significant difference between active and inactive PHHs. In total, 23 of the 27 PFAS compounds studied were shown to have statistically significant hepatic clearance. To further investigate *in vitro* hepatic clearance, those PFAS compounds found to be cleared in the initial screen are being evaluated in more detail via incubations with 50-donor pooled primary human hepatocytes at 0, 15, 30, 60, 90, 120, and 240 minutes. For these 23 PFAS compounds, half-lives ranged from 19 to > 2,400 minutes with corresponding intrinsic hepatic clearance values of 0.04 - 4.9 mL/min/kg. Finally, metabolites for each parent PFAS compounds were predicted using ADMET Predictor/MedChem Studio. This information will be used to assist in the metabolite profiling of the hepatically cleared PFAS compounds to identify, at this time, unknown PFAS metabolites. Additional studies are ongoing to analyze an additional 31 PFAS compounds via LCMS, with the remaining PFAS compounds being analyzed using GCMS.

3170 Risk Assessment of Oral Intake of PFAS from US FDA's Total Diet Study

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Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic compounds that are ubiquitous in the environment due to their widespread use and resistance to degradation. PFAS are detected in drinking water and food products in the United States. In 2019, FDA sampled 91 food products for 16 PFAS compounds, of which 14 samples had measurable concentrations of 5 PFAS. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were detected in 10 samples. Several food products, such as produce and baked goods, had quantifiable concentrations of less commonly studied PFAS, including perfluorohexane sulfonate (PFHxS), perfluoropentanoic acid (PFPeA), and pentafluorobenzoic acid (PFBA). The current assessment aims to calculate human health risk using a Hazard Index (HI) approach resulting from ingestion of PFAS-containing food. Food intake estimates were adapted from both EPA and USDA databases. PFAS intake was compared against EPA reference doses (RfDs) for PFOS and PFOA and against RfD estimates for PFHxS, PFBA, and PFPeA. All PFAS were assumed to be 100% bioavailable from oral ingestion. Meat products (red meat and poultry) had the highest concentrations of PFOS. The estimated daily intake of PFOS was approximately 1.1E-6 mg/kg/day, which is below the RfD of 2E-5 mg/kg/day. The hazard index was estimated as 5.7E-2, or below 1 for PFOS intake from meat. The daily PFOS ingestion from seafood was estimated as 1.3E-7 mg/kg/day. For cumulative meat and seafood, daily PFAS ingestion was 1.3E-6 mg/kg/day. These results indicate that daily PFOS intake from meat and seafood consumption is not associated with an increased risk of adverse health effects. Cumulative consumption of PFOS from other contaminated foods may lead to an exceedance of the RfD and is discussed relative to the FDA diet study data. Further sampling of food products for PFAS will strengthen exposure assessments. Additional studies on atypical dietary intake of meat (e.g., Paleo diet) may help further characterize PFAS oral exposure risk.

3171 Using ToxCast and Reactome to Evaluate Toxicity of PFAS

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment due to their wide use in consumer products, as well as their slow degradation. The environmental fate and human health effects of two commercially successful PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been extensively studied. There is strong evidence that hepatotoxicity, immunotoxicity, and developmental toxicity observed in PFOA/PFOS-exposed rodents involve the activation of peroxisome proliferator-activated receptor-α (PPARα); however, studies in PPARα-null mice suggest that PPARα-independent mechanisms also contribute to hepatotoxicity and immunotoxicity. During the prior decade, manufacturing uses of PFOA and PFOS were phased out as part of global stewardship programs; however, thousands of newer PFAS that are less well-characterized are currently used in consumer products. Consequently, there is the need to better understand the toxicity of newer PFAS to ensure their safety. To this end, this study explored an *in silico* approach to assess toxicity of PFAS. In order to validate this approach, the study focused on PFOA and PFOS, whose toxicity has been well characterized. Specifically, ToxCast was used to gather data available for human liver HepG2 cells, in order to identify genes that are upregulated or downregulated upon exposure to PFOA or PFOS. The identified genes were then analyzed using Reactome, in order to understand which biological pathways are impacted. Subsequently, the results were discussed in the context of the known biological effects of PFOA and PFOS. This study demonstrated that several biological pathways identified through ToxCast-Reactome were found to be impacted in the literature on PFOA and PFOS. Thus, this *in silico* approach could be used in assessing the toxicity of the newer PFAS, as well as other toxicants.

3172 Antigenic, Inflammatory DHP-Lysine Adducts Are Induced by Aldehydes in the Vapor of Both Flavored and Unflavored E-cigarettes and Heated Tobacco Products

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E-cigarettes and heated tobacco products (HTPs) are believed to produce fewer toxic chemicals than traditional tobacco smoke due to the lack of need for combustion. However, we recently demonstrated the presence of several carbonyl compounds in eluates from the mainstream vapor of HTPs (Uchiyama, *et. al.* (2018) *Chem. Res. Toxicol*. 31, 585-593). These carbonyl products cause toxicity through direct adduction to proteins as well as indirect oxidative stress. Oxidative stress further results in the formation of reactive aldehydes including malondialdehydes (MDA). We recently discovered that the antigenic, inflammatory 1,4-dihydropyridine (DHP)-type lysine adducts (M2FA) were produced by the reaction between MDA, formaldehyde, and lysine. These adducts consist of two molecules of MDA, one molecule of formaldehyde and lysine. As vapor from e-cigarettes and HTPs includes various aldehydes and can lead to formation of MDA in the lung, here we tested whether such aldehydes produce complexed lysine adducts in the presence of MDA. Aldehydes utilized in this study include formaldehyde, acetaldehyde, acrolein, methylglyoxal, glyoxal, diacetyl, cinnamaldehyde, and vanillin, all of which are found in the vapor of e-cigarettes and HTPs. Each aldehyde was incubated with 6-ACA, a lysine analog, in the absence or presence of MDA in PBS at 37°C. MDA markedly enhanced the complexity of lysine adduct formation in addition to the formation of very stable products. We detected formaldehyde-, acetaldehyde-, and methylglyoxal-derived DHP lysine adducts. In addition, we found that other aldehydes also likely produce DHP-lysine adducts, as determined by both UV-absorbance and fluorescence properties. The structure of these products will be analyzed via a LC-MS method. In the independent study, interestingly, we found that endogenous DHP-lysine adducts were abundant in type-II alveolar epithelium and bronchial epithelium in normal lung tissues. This result suggests that formaldehyde-derived DHP lysine adducts may be present in surfactant molecules. We now hypothesize that a combination of endogenous and inhaled exogenous aldehydes produce complex DHP lysine adducts, which may lead to chronic lung inflammation and diseases such as chronic obstructive lung disease.

3173 *In Vivo* Inhalation Study for 28 Days to Investigate the Effects of Propylene Glycol, Water Mist, and Their Sequential Combination on Sprague Dawley Rats

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Propylene Glycol (PG) is commonly used as a main ingredient of e-liquids. Although PG is known to cause dehydration, the biological effects of PG are not entirely clear. To investigate such effects, we conducted a 28-day *in vivo* inhalation study using rats exposed to a PG aerosol, water mist, or their sequential combination (PG-Mist). An aerosol with a PG concentration of 1,500 µg aerosol corrected mass (ACM)/L was generated from a vaping device using a smoking machine, and water mists with water concentrations of 5,000 and 15,000 µg ACM/L were generated using a nebulizer. Nine-week-old male Sprague-Dawley (SD) rats were exposed to these conditions for 5 days/week for 1 hour, and then necropsies were performed following 28 days and 14 days of recovery. In addition to these exposure conditions, PG-Mist groups were exposed to alternating PG aerosol and water mist every 30 minutes for 2 hours over 28 days. All results were compared with a filtered air-exposed group. The composition and characteristics of test atmospheres were stable throughout the study period. No relevant effects of exposure to PG aerosol, water mist, or PG-Mist were observed on body weight, hematology, blood biochemistry, bronchoalveolar lavage fluid analysis, or respiratory parameters compared with the filtered air group. In the histopathological examination, some adaptive changes, such as basal cell hyperplasia and metaplasia (graded as low-medium severity) were found locally in the nasal cavity and larynx in not only PG aerosol and PG-Mist groups, but also water mist groups, suggesting that these changes were attributed to particle exposure. Therefore, adverse effects of PG and the effect of PG-induced dehydration were not obviously observed. In conclusion, these results indicate that there might be no clear biological effects of PG inhalation.

3174 The Differential Responses of Human Nasal Epithelial Cells (hNECs) from Nonsmokers and Smokers to "Vaped" Propylene Glycol and Glycerol

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In the United States, over 10 million adults are current e-cig users. Among 18-24 years old e-cig users, 40% are considered to be never smokers. It is not clear, whether and to what extend prior smoking status affects biological responses induced by e-cigs. There is ongoing research on the general effects of e-cig use, with additional focus on the toxicity of specific e-cig components, such as flavoring compounds and nicotine, yet less focus on the humectants, PG and GLY. The goal of this study is to compare the biological effects of the aerosol from the vaped humectants, PG and GLY, on airway epithelial cells from smokers and non-smokers. Differentiated human nasal epithelial cells (hNECs) from smokers and non-smokers were exposed at air liquid interface to the aerosol of the vaped humectants at PG:GLY ratios of 100:0 (PG), 0:100 (GLY), 55:45 (PG:GLY). Each 10 minute exposure consisted of 4 sec puffs every 30 seconds for a total of 20 puffs at a flow rate of 2.5 LPM. Cells were analyzed 24hrs post-exposure. Our data demonstrate that hNECs from non-smokers have elevated secreted MUC5AC and MUC5B in the apical wash in response to vaped GLY and PG:GLY as compared to air while no significant changes in mucin levels were detected in hNECs from smokers. However, pro-inflammatory cytokines, IL-6, IL-8, IL-1β, VEGF, and IP-10 are only elevated in the basolateral supernatants of hNECs from smokers in response to vaped GLY. These data indicate that different PG:GLY ratios induce differential pro-inflammatory cytokine and mucin secretion. In addition, there are also apparent differences in how hNECs from non-smokers and smokers respond to the aerosol of vaped humectants. Together, these data suggest that prior smoking status determines the biological responses induced by inhaling e-cig aerosols.

3175 E-cig Aerosol Induces Lung Dysregulated Repair Response and Extracellular Matrix Remodeling via α7 Nicotinic Acetylcholine Receptor

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Electronic cigarettes (e-cigs) are considered as less harmful alternative to tobacco smoke. However, there is very little evidence on the toxicological and respiratory health effects of e-cig exposure, especially on lung dysregulated repair and extracellular matrix (ECM) remodeling mechanisms. We hypothesized that sub-chronic (one month) e-cig exposure affects the ECM remodeling/dysregulated repair possibly via α7 nicotinic acetylcholine receptor (α7nAChR) in a sex-dependent manner. Adult C57BL/6J mice (WT) and α7nAChR knock out (KO) mice were exposed to e-cig aerosol containing propylene glycol (PG) with or without nicotine (24 mg/mL) for 30 days (2 hrs/day, 5 days/week). Total RNA and protein were isolated from mouse lungs for NanoString and Western blot analysis. Sub-chronic e-cig aerosol exposure with or without nicotine caused dysregulation of several different matrix metalloproteinases such as MMP2, MMP8, and MMP9 in both male and female mice both at the level of protein abundance and gene expression. Further, MMP12 protein abundance was significantly increased in male mice only. Surprisingly, both MMP9 and MMP8 protein abundance were significantly decreased and MMP2 protein levels were increased in both male and female mice exposed to PG alone. Additionally, we found protein abundance of tissue inhibitor of metalloproteinases (TIMP3), a negative regulator of MMP activity, was significantly decreased in female exposed to e-cig with or without nicotine. Furthermore, e-cig aerosol exposure significantly increased the protein levels of COL1A2 but decreased COL1A1 and fibronectin in both male and female mice. The α7nAChR KO mice exposed to e-cig aerosol showed attenuation of COL1A2 and fibronectin in male, but not in female mice. Similarly, protein levels of MMP2 and MMP12 were modulated in male mice. Overall, this study demonstrates that mice exposed to sub-chronic e-cig aerosol containing PG alone can cause significant effects on both repair responses and ECM remodeling processes at the mRNA and protein levels possibly via α7nAChR in a sex-dependent manner. These findings support the long-term respiratory health effects of e-cig exposure leading to altered dysregulated repair responses and ECM remodeling of the lung in a sex-dependent manner. *This work was funded by the NIH 1R01HL135613.*

3176 Systemic Immune and Oxidative Stress Gene Expression Differs in Smokers and Nonsmokers in Response to Flu

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Epidemiological studies have found that smokers are more susceptible to respiratory infections, including influenza virus. Cigarette smoke weakens the immune system by suppressing host defense mechanisms necessary to fight off infections. To understand the mechanisms underlying this immune suppression, we previously analyzed immune responses in the respiratory tract of smokers following exposure to live attenuated influenza virus (LAIV), a cold adapted virus that limits replication to the nasal passageway. Analysis of nasal lavage fluid (NLF) from smokers following inoculation with LAIV showed both reduced expression and responsiveness of inflammatory markers. We propose that cigarette smoke-induced immune suppression is not limited to the respiratory tract and is indicative of broader systemic immune suppression. To test our hypothesis, we analyzed peripheral blood mononuclear cells (PBMCs) from nonsmokers (n=14) and cigarette smokers (n=11) pre- and post-inoculation with a LAIV for cytokines and markers of antioxidant responses. Participants self-reported smoking status, which was verified by tobacco use diaries and urine cotinine/creatinine ratios. We extracted RNA from PBMCs and assessed them for changes in expression of immune and antioxidant enzyme genes with known functions during antiviral host defense using qPCR. Targets included inflammatory genes *IL-6* and *CXCL10* and antioxidant enzymes *HO-1* and *NQO1*, all of which are important mediators in orchestrating influenza-induced antiviral host defense responses. We analyzed our data using a mixed-effect model followed by a Fisher's LSD post-hoc test. Our findings include baseline, pre-LAIV sex-differences in circulating immune gene expression and tobacco use-dependent responses to LAIV. Male smokers had lower *IL-6* expression than nonsmokers and female smokers had higher HO-1 levels than non-smokers pre-LAIV. In response to LAIV, *CXCL10* expression decreased in smokers at day 4 post-LAIV and increased in smokers by day 21 post-LAIV. In addition, *HO-1* levels decreased in smokers at day 4 post-LAIV, while *NQO1* levels increased in smokers at day 4 and returned to baseline by day 21. These results indicate that smoking is associated with alterations in systemic expression of *IL-6*, *CXCL10*, *HO-1*, and *NQO1*, which may be associated with the suppressed antiviral host defense responses seen in smokers.

3177 The Effects of Electronic Nicotine Delivery System (ENDS) on Immune Cell Effector Functions

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Cigarette smoking is a major risk factor for lung cancer. Immune suppression, resulting from chronic inflammation, is suggested to increase the susceptibility of smokers to cancer. Electronic Nicotine Delivery Systems (ENDS) are non-combusted, inhaled tobacco products that are relatively new in the market place and whose long-term health effects are not completely understood. We have previously reported that exposure of human peripheral blood mononuclear cells (PBMCs) with aqueous extracts (whole-smoke condition media, WS-CM) from 3R4F reference cigarettes suppressed agonist-stimulated cytokine secretion and cytolytic killing. In this study, we evaluated the effect of exposure to two marketed ENDS aerosol extracts (aerosol-conditioned media, A-CM) on select immune functions. Natural Killer (NK) cells effector function, as measured by cytolytic killing, was equivalent in PBMCs exposed to either of the ENDS preparations compared to unexposed cells. In addition, the levels of cytokines secreted by PBMCs exposed to either ENDS preparation were comparable to unexposed PBMCs. Collectively, these data show that the tested ENDS aerosol preparations did not adversely impact cytolytic killing or cytokine secretion even at higher doses relative to combustible tobacco product preparations.

3178 Acute Electronic Vapor Product Aerosol Exposure of 3D Human Bronchial Tissue Results in Minimal Gene Expression Changes When Compared with Tobacco Smoke

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Amongst adult smokers, the use of electronic vaping products (EVPs) continues to increase worldwide. This is in parallel with accumulating evidence of the potential reduced toxicity of EVPs and their harm reduction potential compared to combustible cigarette. Advances in molecular biology and the computational sciences now allow for a deeper and more mechanistic understanding of the potential risks associated with cellular exposure to EVP aerosols. In this study, the transcriptomic, functional and cellular perturbations that occur in a fully differentiated 3D *in vitro* reconstituted upper airway human epithelium (MucilAir™) were investigated. We assessed acute exposure to whole EVP aerosol (*my*blu™ blueberry flavour, 2.4% nicotine) with 4-and 48-hours recovery. Results were compared to combustible reference cigarette (Kentucky Reference Cigarette 3R4F) smoke (3.5% whole smoke concentration with an equivalent nicotine dose). Exposure to cigarette smoke resulted in a significant impact on normal barrier integrity and cilia beat frequency at 4 hours compared to both air control and EVP exposure. Proinflammatory cytokine concentrations in culture medium following 48 hours recovery were also significantly increased. EVP aerosol had no observable impact on barrier integrity, cilia beat frequency or proinflammatory cytokines under the conditions of test. RNA sequence and gene set enrichment analysis demonstrated a minimal gene set response to EVP aerosol, whilst exposure to cigarette smoke triggered a clear response in several key disease pathways. In conclusion, an acute EVP aerosol exposure of a 3D *in vitro* reconstituted human lung cell culture had a minimal transcriptomic effect and no functional or inflammatory response when compared to cigarette smoke exposure in this study setup.

3179 Exposure to Pod-Based Electronic Cigarettes Flavors Causes Mitochondrial Dysfunction in Lung Epithelial Cells

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The use of electronic cigarettes has been on the rise in western populations with the use of electronic cigarettes in middle- and high-schoolers being of particular concern. This increase in use and the emergence of vaping associated respiratory illnesses have resulted in certain states, to ban the sale of flavored e-cigarettes, with few exclusions such as tobacco flavors. The health effects of electronic cigarettes are not fully known and we hypothesize that the electronic cigarettes flavors will decrease mitochondrial function and induce mitochondrial superoxide production. Mitochondrial oxidation-phosphory-

lation was measured in lung epithelial cells (Beas2b) on seahorse plates and exposing the plates to air or pod-based e-cigarette aerosols for three 30 minute sessions. Immediately after and 24 hours after exposure, mitochondrial respiration was measured with the cell mito stress test kit using the Seahorse XFp analyzer. Mitochondrial superoxide production was measured in Beas2b cells and exposing the cells to air or pod based e-cigarette aerosols in three 30 minute sessions. Six hours after the last exposure the cells were measured by flow cytometry using Guava Millipore Easycyte-8 instrument. Immediately following the last exposure to JUUL Menthol aerosol resulted in a significant increase in non-mitochondrial oxygen consumption and proton leak compared to air control and also resulted in a decrease coupling efficiency compared to air control. Twenty-four hours following the last cell exposure to JUUL Menthol aerosol resulted in a significant increase in non-mitochondrial oxygen consumption along with a significant decrease in basal respiration, maximal respiration, and spare capacity of the mitochondria compared to air controls. Exposure to JUUL menthol aerosol resulted in an increase in MitoSox high and Annexin V low cell populations and a decrease in MitoSox low and Annexin V low cell populations compared to air. Our results indicate that exposure to pod-based e-cigarette flavors result in mitochondrial dysfunction and the potential to increase mitochondrial superoxide production. *Supported by T32 ES007026, NIH 1R01HL135613, and CTP NCI U54CA228110.*

PS 3180 Elemental Leaching from Electronic Cigarette Coils over Time

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Electronic cigarettes (e-cigarettes) were first introduced to the US market in the mid-2000s. In 2018, e-cigarette sales were approximately $3.6 billion. This industry has greatly influenced nicotine use and possible addiction in adolescents. E-cigarette use in high school students has increased from 1.5% to 16% from 2011 to 2015, surpassing the prevalence of conventional cigarette use. While the majority of studies focus on flavorings and/or nicotine effects for e-cigarettes, the effect of elements leaching from the metal coils that heat e-cigarette solutions has been largely overlooked. Studies have shown exposure to metals contributes to breathing difficulty, kidney dysfunction and even cancer, depending on the element. A number of e-cigarettes use coils made from stainless steel containing silicon (Si), chromium (Cr), nickel (Ni) and molybdenum (Mo). Our objective was to determine the degree and type of elemental leaching that might occur from a heated e-cigarette coil into the aerosol. A third-generation e-cigarette device was used that heats the coil to 232°C, to produce an aerosol. The aerosol was generated using an equal ratio of propylene glycol (PG) and vegetable glycerin (VG) solvent solution (50:50 PG/VG). The aerosol created was introduced into a chamber. The vaping device was set at a puff time of 3 seconds twice a minute over a 3-hour period (3 seconds/puff, 2 puffs/minute) for a total of four consecutive days. Aerosol was collected on Teflon filters with characterization done by X-Ray Fluorescence (XRF). Two collection times were selected: after 720 puffs (day 2) and after 1440 puffs (day 4). XRF analysis detected the presence of Si and aluminum (Al) in the aerosol. Si is a metal normally present in stainless steel. The presence of aluminum in the aerosol might be due to suboptimal stainless-steel manufacturing practices. Thus, the presence of both metals would appear to be via leaching from the coil. A large amount of chlorine (Cl) was also detected by XRF. The origin of the chlorine is unclear, since it should not arise from the stainless-steel coil or come from the pure PG/VG solution. One possible source might be from the wick used in the vaping device. These findings demonstrate the composition of e-cigarette vaping is not simply the product of aerosolized PG/VG, but rather, other components arising from the device, including metals found in the coil and released by heating, thus leaching into the vaping/aerosol mixture.

PS 3181 Biological Effects of Progressive Exposure to Vapor from a Third-Generation E-cigarette Device

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E-cigarettes are popular devices that deliver nicotine to users by heating e-liquid: a mixture of propylene glycol (PG), vegetable glycerin (VG), nicotine, and artificial flavors. Since these devices are relatively new, the long-term health effects of inhaling e-cigarette vapor are largely unknown. To investigate the pulmonary effects of these devices, a novel 3rd generation e-cigarette exposure system was created to expose mice to e-cigarette vapor. This system features precise control of temperature and power (wattage) settings on the device to ensure puff consistency. The system also controls the frequency and duration of each puff, as well as the concentration of e-cigarette aerosol generated. A total of 48 BALB/c mice were placed in the e-cigarette exposure chamber or a filtered-air control chamber for 3 hours per day, 5 days per week for 2 weeks. During the 3 hours, 3-second aerosol puffs were generated twice per minute. Daily aerosol and nicotine concentrations were measured using gravimetric filters and XAD-4 cartridges, respectively. Necropsies were performed immediately following exposure on days 1, 3, 5 and 10 (n = 6/time point). Serum was collected for nicotine and cotinine measurement, and bronchoalveolar lavage fluid (BALF) was collected to determine total cell number, viability and differentials. Right lung lobes were flash-frozen for qPCR and ELISA, and the left lung was inflation-fixed with 4% paraformaldehyde for histological analysis. Over the 10-day exposure, average e-cigarette aerosolized nicotine concentrations were 16 ± 1 mg/m^3, resulting in serum nicotine concentrations of 133 ± 46 ng/mL and serum cotinine levels of 712 ± 302 ng/mL. These serum values are consistent with published serum nicotine and cotinine levels found in active tobacco smokers. Interestingly, both serum nicotine and cotinine were highest on day 3, with values on day 5 and 10 decreasing to levels similar to day 1. This is despite exposure conditions being consistently maintained throughout the 10-day period. E-cigarette exposure significantly increased total cell numbers in BALF on day 1 (P= 0.028), but did not increase to a statistically significant degree on days 3, 5 or 10. Macrophages were significantly increased compared to control on day 5 (P=0.026) with a trend of increased macrophages at all other time-points. These data suggest that exposure to e-cigarette vapor may alter the cellular infiltrate of the lungs which could dysregulate the pulmonary immune system as a whole.

PS 3182 Zinc at Concentrations in the Electronic Cigarette Aerosols Induces Stress in Human Bronchial Epithelial Cells

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Several studies have reported elevated zinc concentrations in aerosols from electronic cigarettes (ECs); however, the effect of inhaled zinc in EC aerosols on the respiratory system has not been investigated. The components, including metals, in EC aerosols are of interest given the recent increase in hospitalizations and deaths due to VAPI (vaping-associated pulmonary illness). In this study, we investigated the effect of zinc on cultured human bronchial epithelial cells (BEAS-2B) using a range of concentrations of zinc found in EC aerosols. The highest concentration of zinc decreased mitochondrial reductase in the MTT assay. In addition, zinc produced a concentration dependent inhibition of cell proliferation in live cell imaging experiments. We then used Fluozin3™, a fluorescent zinc probe, to track the movement of zinc inside cells. Within 24 hours of treatment, numerous vesicles containing zinc were present in the cytoplasm. To determine if uptake was via endocytosis, cells were incubated for 24 hrs with Dextran- Alexa555™ and zinc, and then incubated in zinc probe for 30 minutes. The dextran and zinc vesicles were co-localized, supporting endocytotic uptake of zinc. Endocytosis was validated using Dyngo4A®, an endocytosis inhibitor. Zinc was tracked through the endocytotic pathway using markers for late endosomes (Rab7), multivesicular bodies (TSG101), and exosomes (CD63). Exosomes were isolated from culture medium using an ExoQuick kit and sized and quantified using a NanoSight 300. Exosomes were lysed in RIPA buffer and released zinc, which was quantified using the zinc probe. After 24 hrs of zinc treatment, there was a significant increase of IL6 in the culture medium as determined using an ELISA. In addition, after 48 hrs, an increase in oxidized glutathione was observed in the treated cells. Similar uptake of zinc was observed when cells were exposed at the air-liquid interface (ALI) to $ZnCl_2$ using a cloud chamber. Our data showed that human bronchial epithelial cells in submerged culture are stressed by concentrations of zinc found in EC aerosols. Cells rapidly internalized excess zinc and packaged it into exosomes, which were secreted, while at the same releasing an inflammatory cytokine and showing evidence of oxidative stress. Chronic stress induced by zinc exposure may damage the respiratory epithelium and could lead to disease and/or contribute to VAPI.

PS 3183 Analysis of Popular Disposable E-cigarette Liquids Using Gas Chromatography-Mass Spectrometry

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For the past ten years, there has been a shift in the way people use nicotine, from cigarettes to electronic smoking devices (e-cigarettes). The recent surge of use also comes from teenagers and adolescents, with reports from the Food and Drug Administration (FDA) claiming that use of popular devices rose from 11.7% in 2017 to 20.8% in 2018 in high schools (FDA 2018). This in an alarming 78% increase that affects over 3.05 million American high school students. Not only is it effecting high school age students, the FDA claims that middle school age use has increased from 3.8% in 2017 to 4.9% in 2018. This

4.9% translates into about 570,000 middle school aged students (FDA 2018). The focus of this research is on four different brands of disposable e-cigarette devices and cartridges: STIG, EONSMOKE, FLAIR, and JUUL. The STIG device was the only one that was a disposable unit rather than a cartridge. It is known that these cartridges contain a highly concentrated solution of salt-based nicotine (5%), as well as other chemicals such as, propylene glycol, vegetable glycerin, benzoic acid, and come in a variety of flavorings. However, it is unclear if there are additional chemicals within the cartridges that may have a harmful impact on the user. To assess that we utilized solid-phase microextraction (SPME) gas chromatography-mass spectrometry (GC-MS) to analyze the solution in the cartridges. We analyzed each brand at full strength, 66% and 50% and then compared the spectra between the brands. There were trace amounts of many different chemicals in each solution, however, we focused on the conserved compounds found within the cartridges and absent from ingredient lists or warning labels. Those include: palmitic acid, myristic acid, acetophenone, stearic acid, bisphenol A, and 2,2-dimethoxy-2-phenylacetophenone. Bisphenol A has been shown to moderately decrease scores on two key lung functional measurements in children, FEV1/FVC method that measures airway obstruction and the FEF2575 method that measure small airway functions (Spanier, Adam J 2014). The FEF2575 is the forced expiratory flow at 25%-75% pulmonary volume method, while the FEV1/FVC is the forced expiratory volume in one breath (FEV1) divided the total amount of air exhaled during FEV1 (Thompson, Gregory 2018; Marseglia, Gian Luigi 2007). Stearic, myristic, and palmitic acids are respiratory and skin irritants that are also known benefactors in the development of acute respiratory distress syndrome (RDS) and pneumonia through the alteration of pulmonary surfactant (Schmidt, Reinhold 2001). The chemical 2,2-dimethoxy-2-phenylacetophenone is known to induce apoptosis in MRC-5 cells in the lungs as well as cytotoxicity in human embryonic fibroblasts (Kawasaki, Yoichi 2014). Acetophenone is another chemical that is a known skin and eye irritant that could also be a contributor to respiratory distress as well as irritation. *(McAuley, David B 1984.)*

3184 *In Vivo* Genotoxicity Testing of Aerosolized ENDS E-Liquids

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The US Food and Drug Administration (FDA) guidance on premarket tobacco applications for e-vapor products recommends toxicity assessment including *in vitro* genotoxicity. As part of the hazard assessment, we tested three different e-liquids (Formulations-I, II, and III, with nicotine levels ~ 2.1%) according to standard OECD *in vitro* cytotoxicity (NRU TG129) and genotoxicity (Ames TG471 and micronucleus [MN TG487]) guidelines. None of e-liquids were mutagenic (in Ames assay) or cytotoxic (in NRU assay) under tested conditions, however they showed a weak but statistically significant dose-related increase in MN, resulting in positive findings in the MN assay. A follow-up *in vivo* genotoxicity inhalation study (a combined MN and Comet assay) was conducted according to ICH guidance S2(R1). Male Sprague Dawley (SD) rats were exposed to filtered air (negative control) or e-liquid aerosols via nose-only inhalation for up to 6 hr/day for 4 consecutive days. The capillary aerosol generator (CAG, 275°C) was used to generate the aerosols with total particulate matter (TPM) within the respirable size range for rodents (MMAD 1-1.7µm and GSD 1.5-2.0). The maximum exposure concentrations (up to 8 mg/L total particulate matter [TPM]) were tested based on their respective maximum tolerated dose in SD rats. The study included concurrent positive controls for MN (cyclophosphamide) and comet assay (ethyl methane sulfonate). Blood was collected immediately after the last exposure for analysis of nicotine and cotinine as biomarkers of exposure. At necropsy, bone marrow samples were collected for MN, and liver, lung, and nasal tissue samples were collected to evaluate DNA damage using the Comet assay. The plasma nicotine (575-5830 ng/mL) and cotinine (607-8210 ng/mL) levels increased with increasing aerosol exposure. There was no increase in the induction of micronuclei formation (% MN) in the bone marrow and DNA damage (% tail DNA) in the liver, lung or nasal tissue. In conclusion, under *in vivo* assay conditions, all tested e-liquids were negative for genotoxicity, implying no biological relevance of the *in vitro* genotoxicity signals for the three e-liquid formulations tested.

3185 Evaluation of the Cytotoxic and Genotoxic Potential of Select Flavor Additives Used in Cigarettes

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Flavor additives are commonly used in tobacco products. However, their contribution to the inherent toxicity of cigarette smoke remains unclear. The present study was designed to investigate the potential cytotoxicity, genotoxicity, and mutagenicity associated with ten additives: benzaldehyde, black licorice extract (LE), ethyl vanillin (EV), glycerol, hot cocoa, 4-hydroxyphenylbutanone (HPB), L-menthol (LM), peppermint oil (PO), piperonal, and sucrose. Lung epithelial (HBEC4 or A549) and cardiac myoblast (H9c2) cells were exposed to these additives at defined dose ranges in their native form and in combinations as an aerosol (at the air-liquid interface) following addition to a Kentucky reference (3RF4) cigarette. The Canadian Intense (CI) and the International Organization for Standardization (ISO) smoking regimens were used to generate the aerosol. Cytotoxicity was assessed using the neutral red uptake [NRU], methylthiazol tetrazolium [MTT], and lactate dehydrogenase [LDH] assays. Genotoxicity was examined using the Comet and micronucleus [MN] assays while mutagenicity was assessed using the Ames assay. In their native form, six additives (benzaldehyde, EV, HPB, LM, PO, and piperonal) showed significant cytotoxicity in both cell lines with the NRU and MTT assays. Regarding aerosol exposure, cigarettes containing defined combinations of additives yielded IC50 values in cytotoxicity assays that were not different than reference cigarette with either smoking regimens. The H9c2 lines appeared more resistant to the cytotoxicity of the cigarette smoke than HBEC cells. In the Ames assay, significant mutagenicity was largely seen in the TA100 strain with and without S9 and the YG1024 strain with S9. The CI smoke regimen induced more mutagenicity than the ISO regimen. Only the custom cigarettes containing LM and LE with the YG1024 strain +S9 exposed using the CI regimen, and benzaldehyde with TA100, and EV with TA97a and TA98 showed a significantly elevated dose response from the Kentucky reference cigarette. The IC50 values in the MTT and LDH assays were much lower in cigarettes containing LM compared to the other additives or reference cigarette. Although all custom cigarettes caused a significant dose-dependent increase in the Comet assay, a dose response that differed significantly from the Kentucky reference cigarette was seen only for EV at the highest dose with the CI regimen in HBEC4 cells. Under the study conditions, the tested additives either alone or in combination demonstrated increased cytotoxicity and genotoxicity, thereby suggesting a contribution to the toxicity of cigarette smoke.

3186 A Structure-Based Grouping Approach for Predicting Biological Activity of Flavor Ingredients Contained in E-vapor Products

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The use of flavors is important for development of e-vapor products. However, many flavors do not have safety or toxicity data, especially for inhalational administration. Considering the large number of available flavors and potential combinations, standard toxicological testing of individual ingredients is not always feasible, as it may require extensive animal testing. We developed a structure-based approach to select a total of 38 flavor group representatives (FGRs) from more than 200 commonly used neat flavors based on the concept that structurally-related compounds have comparable metabolic and biological activities (as outlined in the European Commission Regulation [EC] No. 1565/2000). We propose that the representative FGR mixtures could be tested in inhalation studies to support the toxicological assessment of all structurally-related individual flavors. Prior to FGR selection, the available toxicological data were reviewed for each flavor (e.g., NOAEL, LC50, and LD50). If no experimental data were available, toxicological predictions (p) were made for key endpoints by using the TOPKAT software (pIrritancy, pCarcinogenicity, pChronic LOAEL pDevToxicity, and pCramerClass). In addition, cytotoxicity potential of the flavors (XCelligence) was experimentally tested by real-time cellular analysis, and the biological impact of a subset of the most cytotoxic substances was evaluated by high-content screening (HCS). HCS data for the remaining flavors were obtained by using a predictive model based on pCramer, pIrritancy, pChronicLOAEL, pExpCarcinogenicity, and pXCelligence (pToxPiHCS). For objective selection of FGRs, flavors within each group were ranked based on pLD50, pDevToxicity, PToxPiHCS, pChronicLOAEL, and pIrritancy scores. The average rank for each flavor ingredient was then computed to generate the final ranking upon which the predicted "worst case" FGRs were selected for each structural group. The resulting FGRs could be tested alone or in combination as mixtures using *in vitro* and *in vivo* toxicity testing

and the results would help deriving acceptable levels for their use in e-vapor products. Once validated, this process will facilitate the read-across of new flavors according to structural groups.

3187 A Structure-Based Grouping Approach to Evaluate Toxicity of e-Vapor Flavor Ingredients: Five-Week Inhalation Study in A/J Mice

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Flavor ingredients are widely used in e-cigarette products. Most flavors are generally recognized as safe for use in foods. However, limited toxicological information is available to evaluate the potential hazard of flavors delivered via inhalation. Considering the number of available flavors and the numerous potential flavor combinations, toxicity testing of each individual compound or formulation is not always feasible. Therefore, we selected the Flavor Group Representatives (FGR) based on structural grouping: a total of 38 representatives were selected from ~200 common flavors, based on known and *in silico* predicted toxicological information. In this study, the selected FGRs were combined to create a potential "toolbox" flavor mixtures and subjected to *in vivo* inhalation testing using A/J mice. The study is a dose range finding study with emphasis on subacute toxicity and respiratory tract irritation and inflammation. The results from the study may be used to select the appropriate concentrations of flavor mixtures for future chronic inhalation study. A/J mice were whole-body exposed to fresh air (Sham), aerosol from base (propylene glycol (PG) and vegetable glycerin (VG) with 2% nicotine (N)), aerosol from flavor mixtures (PG, VG, 2% N with up to 18% of flavors (F)), or to mainstream smoke (MS) from the 3R4F reference cigarette for 6 hours per day, for 5 days per week, for 5 weeks. The aerosols was well tolerated by the mice, without signs of severe acute toxicity post-exposure. Exposures to the flavored aerosols, even at the highest flavor concentration, did not cause lung inflammation based on the lack of immune cell infiltrate in the bronchio-alveolar lavage fluid and histopathology evaluation. By contrast, exposure to MS resulted in lung inflammation and also moderate to severe adaptive changes in the nasal and laryngeal epithelia. Most of upper respiratory track changes were absent in mice exposed to flavored e-vapor or significantly less severe than in the MS-exposed mice. In summary, the tested flavor concentrations did not result in severe subacute toxicity or respiratory tract irritation/inflammation and were considered suitable for use in future chronic inhalation study in A/J mice.

3188 Comparison of Biological Effects between Reference Cigarettes 1R6F and 3R4F: A 28-Day *In Vivo* Inhalation Comparative Study

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Kentucky reference cigarettes have been widely used as comparative cigarettes for *in vitro* and *in vivo* toxicological research of tobacco-related products. The 1R6F Kentucky reference cigarette (1R6F) has been made available because of the depleting supply of the 3R4F Kentucky reference cigarette (3R4F). Recently, an *in vitro* comparative study of 1R6F and 3R4F was reported, but these is no *in vivo* toxicological research comparing those reference cigarettes. Therefore, to investigate differences in the *in vivo* biological effects of both reference cigarettes, we conducted a 28-day *in vivo* inhalation toxicity study with reference to the OECD Test Guideline 412 (OECD, 2018). In this study, male Sprague Dawley rats were exposed to mainstream cigarette smoke (MCS) from 1R6F or 3R4F (330 µg wet total particulate matter/L) by nose-only inhalation for 6 h/day, 5 days/week for 4 weeks. The control group was exposed to filtered air under the same exposure condition. In addition to the endpoints provided by the OECD TG412, we performed lung function measurements using the flexiVent® system and transcriptomic analysis of lung tissues. As a result, 1R6F and 3R4F groups were confirmed to show effects related to MCS exposure in any biological parameter listed in the OECD TG412 and in lung function measurements, compared with the control group. In addition, no significant differences were found in the effects of 1R6F and 3R4F groups. In transcriptomic analysis, the number of differentially expressed genes in the lungs almost corresponded between 1R6F and 3R4F groups after 4 weeks of exposure. Furthermore, the results of Gene Ontology enrichment analysis suggested that the gene expression profile of 1R6F group was similar to that of 3R4F group. On the basis of these results, we indicated that 1R6F can be used as a comparative cigarette as well as 3R4F for *in vivo* toxicological research.

3189 Effects of an Aqueous Extract of Aerosol from a Heated Tobacco Product on Reconstructed 3D Human Epithelial Tissues

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Next generation tobacco products, including heated tobacco products (HTPs), are becoming increasingly available worldwide. HTPs heat a tobacco without combustion. Hence, the resulting aerosol theoretically contains reduced amounts of harmful and potentially harmful constituents resulted from combustion. However, the biological effects of aerosol from HTPs on the respiratory tract are not fully understood. In the current study, we investigated the biological effects of aerosol from one of our HTPs, prototype tobacco vapor product (PTVP), on reconstructed three-dimensional (3D) human airway epithelial tissue in comparison with that from the 1R6F reference cigarette. Each aqueous extract (AqE) of 1R6F and PTVP was prepared by bubbling the generated aerosol into medium. Then, the AqEs of 1R6F and PTVP were diluted to each concentration ranging from 50 to 200 puffs/L and 200 to 10000 puffs/L, respectively. After 24 h of exposing the tissues to each AqE, we analyzed the cytotoxic effect [adenylate kinase (AK) activity], ciliary function [ciliary beating area (CBA)], inflammatory response [interleukin (IL)-8 secretion], and global gene alteration [transcriptome]. The tissues exposed to AqE of 1R6F showed a significant increase in AK release at 200 puffs/L, while it was observed at a higher concentration (only at 10000 puffs/L) when AqE of PTVP was exposed to the tissues. A significant decrease in CBA was also observed in the tissues exposed to AqE of 1R6F at 200 puffs/L, whereas no significant change was observed in the tissues exposed to AqE of PTVP at any concentration. Furthermore, the AqE of 1R6F induced a dose-dependent and significant increase of IL-8 secretion, while no significant change was observed in the tissues exposed to AqE of PTVP at any concentration. In the transcriptomic analysis, fewer differentially expressed genes (DEGs) were detected in the tissues exposed to AqE of PTVP than 1R6F at comparable nicotine concentration. We identified 483 of DEGs at a sub-toxic concentration (10000 puffs/L) in AqE of PTVP exposure, and those DEGs were mostly related to oxidative stress response pathways (e.g., NRF2-mediated oxidative stress) which are well-known cigarette smoke-inducible pathways. Taken together, although the high amount of PTVP aerosol exposure may elicit similar biological processes as cigarette smoke exposure, our results suggest that the PTVP have weaker biological effects than cigarette smoke.

3190 Cigarette Smoke-Induced Autophagy Is Regulated by FOXO Transcription Factors

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Chronic obstructive pulmonary disease (COPD) is a progressive and life-threatening disease with the major risk factor being exposure to cigarette smoke. Cigarette smoke (CS) contains more than 5000 chemicals which exposes lungs to high concentration of free radicals and bioactive chemicals. Oxidative stress results in DNA damage, degradation of proteins and cell death resulting in acute lung injury. The lung damage in COPD is irreversible with currently no available therapy helpful in regressing the damage. Therefore, further understanding about the disease pathogenesis in terms of molecular mechanisms associated with cellular damage during CS exposure is highly warranted. Earlier studies have demonstrated a functional role of autophagy in COPD. Autophagy is a lysosomal degradation process that clears off damaged organelles and misfolded or aggregated proteins as a critical cell survival mechanism in response to stress. Expression and activation of autophagy related proteins is regulated by Fork head box class O (FOXO) transcription factors. Our preliminary findings revealed CS-extract mediated induction of inflammatory responses and regulation of transcription factors FOXO3a and FOXO1 in human lung adenocarcinoma cells with type II characteristics (A549). We thus hypothesized that FOXO transcription factors play a vital role in regulating CS-induced inflammation and autophagy. To test our hypothesis, we challenged A549 with cigarette smoke extract (CSE) for 24 hr. Our findings revealed significantly reduced expression of FOXO3a at both transcriptional and translational level in CSE-exposed A549 cells as compared to control. To further elucidate the role of Foxo3a in CSE-induced autophagy we transfected A549 cells with SiRNA-scrambled or SiRNA-FOXO3a followed by challenge with CSE. We first determined the effect of FOXO3a knockdown on the production of cytokines/chemokines and observed that knockdown of transcription factor augments CSE-induced IL-6, CCL-2 and IL-8 production by A549 cells. Moreover, we also observed FOXO3a mediated transcriptional regulation of several autophagy (LC3B, ATG4, ATG16 and ATG12) and antioxidant (MnSOD and catalase) genes in CSE-challenged A549 cells. Next, we performed chromatin immunoprecipitation assay to determine interaction between FOXO3 and autophagy related genes. The qPCR analysis showed significant increase in the binding of FOXO3a transcription factor on the promoter regions of LC3B, ATG12, GABARAL1 and ATG4 in CSE-challenged cells.

Further experiments are in progress to define the role of FOXO3a and FOXO1 in CSE-induced inflammation and autophagy. Overall, our results will provide important information about the therapeutic targets for the management of CS-induced inflammation/pathologies.

3191 Cigarette Smoke Exposure Exacerbated Silica-Induced Pulmonary Toxicity

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Smoking, an avoidable life style factor, may modify the lung response to silica exposure including cancer and silicosis. Studies have shown that smoking induces oxidative stress and inflammation, etiological factors involved in the development and progression of cancer and silicosis. Nevertheless, the precise role of cigarette smoke (CS) exposure on the lung response to silica exposure and the underlying mechanisms are unclear. Therefore, the objectives of the present study were to determine the role of CS on lung response to silica exposure and the underlying mechanism. Male Fischer 344 rats were exposed by inhalation to air, crystalline silica (15 mg/m^3, 6 hrs/day, 5 days), CS (80 mg/m^3, 3 hrs/day, twice weekly, 6 months), or silica (15 mg/m^3, 6 hrs/day, 5 days) followed by CS (80 mg/m^3, 3 hrs/day, twice weekly, 6 months). The rats were euthanized 6 months following initiation of the exposures and lung response parameters including, lactate dehydrogenase (LDH) activity, oxidant production, cell counts, and cytokines in broncho-alveolar lavage (BAL) were assessed. Silica exposure resulted in significant lung toxicity as evidenced by lung histological changes, enhanced neutrophil infiltration, increased LDH levels, enhanced oxidant production, and increased cytokine levels. The CS exposure had only a minimal effect on the toxicity parameters. However, the combined exposure to silica and CS caused a significant increase in lung response, compared to silica or CS exposure alone. For example, CS or silica exposure alone resulted in neutrophil infiltration 5 and 150 times, respectively, compared to the air-exposed controls. The combined exposure to silica plus CS, on the other hand, caused a neutrophil infiltration that was 500 times higher compared to air controls suggesting a synergistic effect of silica and CS on lung toxicity. Global gene expression changes detected in the rat lungs correlated with the toxicity. Bioinformatic analysis of the gene expression data demonstrated significant enrichment in functions and pathways relevant to silica exposure which correlated with the lung toxicity. Unique pathways relevant to lung response to silica exposure, for example disruption of circadian rhythm signaling, were detected in the rat lungs exposed to silica and CS. Collectively our data demonstrated an exacerbation of silica-induced lung toxicity by CS exposure and the molecular mechanisms underlying the exacerbated toxicity.

3192 Bioinformatics Approach Unravels Toxic Effects of Waterpipe Tobacco Smoking

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Waterpipe tobacco smoking (WTS), in Middle East known as shisha or narghile, is becoming more popular in Western societies, particularly among young people as an alternative form of tobacco use other than traditional cigarettes. The health risk associated with WTS is highly underestimated, despite the fact that waterpipe tobacco use is associated with greater carbon monoxide, similar nicotine, dramatically more smoke exposure and some of the same toxicants as cigarette smoking. The aim of this study was to investigate toxicities induced by light-use WTS. We analysed gene expression data (Walters MS *et al*, 2017) from small airway epithelium of waterpipe tobacco smokers and WTS impact on pathological changes and affected pathways. 282 signature genes (<.05, >.5) were detected from the whole genome analysis, as significantly dysregulated after exposure to WTS., By manually annotating and processing molecular information from the publicly available data (PubMed articles and FDA reports), we created a computational model of biological pathways describing cellular processes in human respiratory tissues and made the information computable. WTS exposure induced genes involved in immune responses, G-protein coupled receptor signalling, oxidative stress and mRNA regulation of translation. In addition, we annotated data about WTS toxic components (such as the ones from Hoffmann's list, Hoffmann D *et al,* 1998) and generated a comprehensive database of known side effects and protein targets of these toxicants. By applying bioinformatics analysis tools to gene expression data and combining with toxicants data, we have identified several pathologies that affect respiratory and circulatory system. We generated mechanistic hypothesis for each of these pathologies, supported by current knowledge. Together, these data indicate that even light-use waterpipe tobacco smoking is as much harmful as traditional cigarettes smoking, and may damage respiratory system.

3193 Toxicological Evaluation of E-vapor Aerosols Using *In Vitro* Regulatory Cytotoxicity and Genotoxicity Assays under Air-Liquid and Air-Agar Interface Conditions

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Users of electronic nicotine delivery systems (ENDS) inhale generated aerosols; therefore, it would be ideal to conduct *in vitro* toxicity testing using systems that mimic direct aerosol exposures to the apical surface of cells or bacteria, i.e., air-liquid interface (ALI) or air-agar interface (AAI). Here we evaluated a cig-a-like device with base formulation (containing humectants and 4% nicotine) and three commercially available cig-a-like ENDS (containing humectants, varying flavors and a range of nicotine concentrations (2.4%-4.8%)) using the Vitrocell®- 24/48 [ALI] and Ames 48 [AAI] exposure modules and regulatory cytotoxicity (neutral red uptake (NRU)) and genotoxicity (Ames) assays. The lung adenocarcinoma cell line, A549 was used for the cytotoxicity assay and was exposed to either air or to e-vapor aerosols at varying puff numbers (50-400 puffs). For the Ames assay, 5 *Salmonella* strains (TA98, TA100, TA102, TA1535 and TA1537) were used and exposed to either humidified air or to 400 puffs of aerosol from each test article. Aerosols were generated using a modified CORESTA CRM 81 puffing regimen (55ml puff volume, 5 sec puff with 30 sec interval). Concentration of the deposited nicotine in the insert was measured as marker of exposure. In the NRU assay, the base formulation was cytotoxic only at 400 puffs (39.1±30.1 % viability), while all three tested e-vapor products showed a concentration dependent cytotoxicity with the estimated IC50 ranging from 72-146 puffs. In the Ames assay, the base formulation and two of tested e-vapor products were found to be negative, however, one e-vapor product was positive in strain TA1535 (>18-fold increase above air control) following exposure to 400 puffs of e-vapor aerosol. The study was repeated with varying puff numbers (50-400) using strain TA1535, wherein a concentration dependent increase in response was observed in comparison to air treated group. In summary, the employed ALI and AAI *in vitro* testing conditions were able to detect varying degrees of cytotoxic and genotoxic hazard in e-vapor aerosols, demonstrating the potential use of direct aerosol testing as part of the toxicological evaluation of e-vapor products.

3194 Thirdhand Smoke Adhesion to and Removal from Indoor Fabrics: Factors Affecting Human Exposure and Remediation

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Thirdhand smoke (THS) consists of residual tobacco smoke that settles and remains on indoor surfaces after smoking has ceased. Remediation of these chemicals is important in reducing exposure to THS. In this study, we investigated the affinity of THS chemicals to common household fabrics that are not washed frequently (e.g. draperies and upholstery) and could act as chemical reservoirs. Cotton, terry cloth, polyester, and wool carpet fabrics were washed four times before being placed in a chamber designed for THS exposure smoke exposure. They were then exposed for 1, 6, 12 or 18 months to 696, 1569, 1795, 3617mg of smoke, respectively. THS was extracted from each fabric at a concentration of 0.1g of fabric/mL of PBS, DMSO, or cell culture medium. Extraction media were examined using fluorescence spectroscopy at various wave lengths. Nicotine, nicotine alkaloid and tobacco specific nitrosamine (TSNA) concentrations in extracts were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Affinity of nicotine to terry cloth and polyester was compared by exposing each fabric to 10 mg/ml of nicotine, and quantifying the amount of nicotine recovered after extraction using high performance liquid chromatography (HPLC). Our results showed that THS chemicals in fabric extracts autofluorescence, and fluorescence was proportional to the time and amount of THS exposure received by the fabrics. THS autofluorescence was not detected in extracts from polyester and wool carpet at any of the excitations tested. Nicotine, nicotine alkaloid, and TSNA concentrations were higher in THS extracts from cotton and terry cloth than in polyester and wool carpet. Using fabrics spiked with 10 mg of nicotine, we showed that extraction efficiency was much higher from terry cloth (7mg) than from to polyester (0.11mg). The absorption into and release of THS from fabrics varied with the type of fabric. Human exposure to THS could be influenced by fabric type, and remediation techniques may need to vary depending on the fabrics reservoirs being treated.

3195 Transcriptome Analysis Reveals Lung-Specific miRNAs Associated with Aberrant Mucociliary Clearance Induced by Cigarette Smoke in an *In Vitro* Human Airway Tissue Model

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Exposure to cigarette smoke (CS) is strongly associated with aberrant mucociliary clearance, which may increase risk for developing CS-related diseases, such as chronic obstructive pulmonary diseases and lung cancer. In this study, we employed miRNA-mRNA network analysis and identified miRNAs that may modulate tissue responses to CS in an *in vitro* human air-liquid-interface (ALI) airway tissue model. Cultures were exposed to CS (diluted in 0.5 L/min, 1.0 L/min, and 4.0 L/min clean air) from smoking five 3R4F University of Kentucky reference cigarettes under the International Organization for Standardization machine smoking regimen, every other day for 1 week (3 days, 40 min/day). Transcriptome analyses of CS-exposed ALI cultures identified 5090 differentially expressed genes and 617 differentially expressed miRNAs. A subset of cilia-associated mRNAs and miRNAs was further enriched by gene ontology and miRNA-mRNA network analysis. These findings are consistent with the reduction in cilia beating frequency and ciliary protein expression caused by CS exposure in the ALI cultures. In particular, a time-dependent decrease in the expression of miR-34/449, a conserved miRNA family highly enriched in multiciliated airway epithelia and implicated in motile ciliogenesis, was observed in CS-exposed cultures. Network analysis further revealed that the down-regulation of miR-34/449 by CS may derepress the cell-cycle proteins, which, in turn, can interfere with cilia biogenesis. Similar alterations have been observed in smokers with COPD. Investigating the effects of CS on miRNA expression in ALI tissue models, therefore, may provide not only mechanistic insights, but potential non-invasive biomarkers for respiratory diseases caused by cigarette smoking.

3196 Natural and Synthetic Antioxidants against Cigarette Smoke Extract-Induced Human Bronchial Epithelial Cell Toxicity

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Cigarette smoke (CS) is an aerosol containing thousands of chemical substances which are not identified completely and suggested that there might be more than 5600 components in CS. From a toxicological perspective, the content of CS which is rich in many fat-soluble toxins, mutagens, and carcinogens which provide a basis for fatal outcomes. Over the last decade, researchers are concerned about the mechanisms of CS induced diseases due to its highly toxic content. Also, preventive strategies against CS is a trend research topic as a part of inhalation toxicology and environmental risk assessment. Alternative therapy methods, particularly natural compounds, are still under investigation due to their antioxidant capacity. In the present study, preventive effects of eucalyptol (EUC), curcumin (CUR) and their combination on CS induced toxicity and oxidative stress (OS) on human bronchial epithelium cells (BEAS-2B) have been investigated *in vitro*. Cigarette smoke extract (CSE) was prepared by 3R4F reference cigarette by bubbling smoke into cell culture medium and 20% CSE (v/v) was used for studies. For each experiment, 2 h pre-treatment with EUC (50 μM), CUR (5 μM) and EUC+CUR was followed by 4 h CSE exposure. 1 mM N-acetylcysteine (NAC) was used as positive control. Cell viability was assessed by MTT assay. Intracellular ROS was determined with using DCFDA in flow cytometry. Also, an important marker in cellular defense against OS, Nrf2 level was measured by western blotting. According to results, cell viability is increased with treatments compared to CSE group (70.13% ±7.65). Intracellular ROS induced by CSE was significantly decreased with NAC and EUC pre-treatments. Also, all pre-treatments lead to a decrase in CSE induced MDA level. CAT activity and GSH level increases with all pre-treatments compared to CSE alone. Nuclear Nrf2 level was significantly increased in CSE group as a response to cellular OS. Pre-treatment with NAC and EUC diminish CSE induced nuclear Nrf2 level while CUR itself act as a Nrf2 activator. According to present results, both EUC and CUR have shown protective effects against CSE induced oxidative bronchial epithelial damage and might be a potential remedy against CS related diseases.

3197 Effects of Exposure to Environmental Tobacco Smoke during Perinatal Development on Susceptibility to Infection and Mortality

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The purpose of this study was to examine the effect of perinatal exposure to environmental tobacco smoke (ETS) on primary influenza and secondary bacterial infection in young mice. Perinatal BALB/c mice were exposed to filtered air (FA) or ETS and then randomly assigned into groups. Three FA groups and three ETS groups were challenged with 1) murine-adapted influenza A virus, 2) the human bacterial pathogen *Staphylococcus aureus*, or 3) both virus + bacteria to determine if ETS impacts resistance to viral or bacterial infection. Bronchoalveolar lavage fluid (BALF) was collected 1, 3 or 10 days post-infection to determine cell numbers and types. Total cell number in the BALF of neonates exposed to influenza A or influenza A + S. aureus was significantly increased, compared to FA and ETS control and bacteria only groups. Clinical symptoms and lung tissue inflammation were scored. Neonates exposed to ETS had similar physical signs of infection compared to animals exposed to FA. FA neonatal groups exposed to influenza A or to both influenza A and S. aureus had 50% mortality. In contrast, neonates exposed to ETS and infection experienced 100% mortality by post-exposure day 10. FA and bacteria only groups had no mortality. Neonates exposed to FA or ETS in combination with virus or virus + bacteria demonstrated significantly higher inflammatory responses compared to groups exposed to only FA, ETS, or bacteria. Using immunohistochemistry, lung sections were stained for Club cell secretory protein (CCSP) and analyzed morphometrically to determine the volume of CCSP per surface area of basal lamina. Club cells are non-ciliated epithelial cells lining the airways which produce crucial proteins and immune factors that aid in the protection of the bronchioles of the lungs. Analysis of terminal bronchioles demonstrated a significant decrease in CCSP in mice exposed to either FA or ETS in combination with exposure to virus or virus + bacteria. There were no statistically significant differences in CCSP expression between the FA and ETS treatment groups. We conclude CCSP expression is significantly reduced in response to influenza A infection. A possible correlation between decreased CCSP levels and viral infection may explain the 100% mortality found in ETS-virus and ETS-virus + bacteria exposed groups in contrast to FA-virus and FA-virus + bacteria demonstrating a return of CCSP levels by day 10, similar to those in groups with no mortality.

3198 Telomere Protection Protein 1 (TPP1) Deletion in Lung Epithelial Cells Augments Environmental Tobacco Smoke-Induced Lung Inflammation

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Telomere attrition is considered a hallmark of aging lung in chronic obstructive pulmonary disease (COPD). We have previously shown that telomere protection protein 1 (TPP1) involved in cigarette smoke (CS)-induced telomeric DNA damage and cellular senescence. TPP1 is one among the key players (shelterin complex: POT1, TRF1, TRF2, Tin2, Rap1, and POT1), that contributes to the maintenance of telomere length and integrity. However, the exact mechanism by which TPP1 mediates protection of shelterin complex against environmental tobacco smoke (ETS) induces lung inflammation, and injury remains unclear. We hypothesized that TPP1 protects against ETS induced telomere DNA damage via the shelterin complex. TPP1-flox and TPP1-CreCC10 (Clara cell-specific TPP1 deletion) mice were exposed to ETS (sub-chronic: 35 days, 100 mg/m^3 total particulate matter). Differential cell counts in the bronchoalveolar lavage (BAL) fluid and inflammatory cytokines were analyzed by flow cytometry and ELISA/Luminex assay, respectively. Additionally, we measured protein abundance of DNA damage, shelterin complex, and cellular senescence markers in the lungs by immunoblotting and histological analysis. Sub-chronic ETS exposure showed increased total cell counts in TPP1-CreCC10 compared to TPP1-flox mice. Neutrophil counts were altered in both TPP1-flox and TPP1-CreCC10 ETS exposed mice compared to air-exposed controls. T-lymphocyte counts were significantly increased in TPP1-CreCC10 compare to air-exposed control. ETS exposure affected inflammatory cytokines (increased: TNFα, IFNγ, IL-5, IL-6, MIP-1β, MCP-1, and eotaxin; significantly increased: IL-4, IL-9, G-CSF, KC, and IL-12p70) in the BAL fluid of TPP1-CreCC10 compared to TPP1 flox air and ETS exposed mice. ETS exposed TPP1-flox and TPP1-CreCC10 mice showed altered expression of DNA damage (γH2AX), shelterin complex (TPP1 and TIN2), and cellular senescence (p21 and p16) markers in the lungs. These data suggest an increased lung inflammation caused by ETS exposure resulted in altered differentiation of naïve T cells and regulation of Th1/Th2 response in TPP1-CreCC10. We conclude that

TPP1 plays a protective role against ETS-induced inflammation and cellular senescence processes that drive COPD pathogenesis. *Supported by the NIH R01 HL135613, R01 ES029177, R21 ES028006, R01 HL133404, and R01 HL137738.*

3199 Exosomal microRNAs Are Novel Circulating Biomarkers among E-cigarette Users, Cigarette, and Waterpipe Smokers

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Electronic cigarettes (E-cigs) recently introduced and gained popularity in US and throughout world. There are severe pulmonary illnesses have been reported in users who used nicotine or cannabis extract in E-cigs. Cigarette and Waterpipe tobacco smoking causes various acute and chronic health effects including cardiovascular disease, chronic bronchitis and cancer. Exosomes are extracellular vesicles of lipid carrying various types of biological materials including RNA in various bodily fluids. MicroRNAs are present in higher concentration in whole RNAs, play a role in various physiological functions and diseases. No study has been done to identify and characterize the plasma-derived microRNAs in E-cig users, waterpipe smokers, and compared with cigarette smokers. The comprehensive analysis of exosomal microRNA expression profiles and their biological functions were performed in plasma samples from normal, E-cig users, waterpipe, and cigarette smokers groups. Our results show upregulation of 17 and downregulation of 5 microRNAs in E-cig users in comparison with non-smokers. Upregulated hsa-miR-30c-5p, hsa-miR-125b-5p, hsa-miR-423-3p, hsa-miR-21-5p, hsa-7f-5p are involved in inflammation, cell proliferation, and epithelial-mesenchymal transition. Downregulated hsa-miR 451a, hsa-miR-10b-5p, hsa-miR-30e-5p are associated with proliferation and inhibition of apoptosis. The altered expression of microRNAs in E-cig users affects several physiological functions, such as biological pathways (erbB receptor signaling network, trail signaling pathway, integrin family cell surface interactions, endothelins pathway) and molecular functions (transcription factor activity, extracellular matrix structure constituent, metallopeptidase activity). Further, significant changes in transcription factors (EGR1, SP1, SP4, ZFP161, POU2F1, NFIC) were also observed. The comparative analysis of microRNA expression and functional characterization of all smoking groups will be presented. Thus, E-cig users and all smoking groups have alteration in plasma exosomes microRNAs expression that may be useful as biomarkers, which are involved in pathophysiology of cardiopulmonary system. *Support by NIH 1R01HL135613 and NIH FDA CTP U54CA228110.*

3200 Menthol Induces Oxidative Stress and an Inflammatory Response in Human Lung Cells Exposed in Both Submerged Culture and at the Air-Liquid Interface

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Menthol is a commonly used flavor chemical that is found in many tobacco products, including electronic cigarettes (ECs). In spite of its widespread use, relatively little is known about how menthol affects the respiratory epithelium when inhaled during smoking or vaping. The purpose of this study was to characterize the effects of menthol on bronchial epithelium using three *in vitro* platforms: submerged culture, VITROCELL cloud chamber that provides air liquid interface (ALI) exposure without solvents nor heating, and a Cultex ALI exposure system the delivers aerosol equivalent to that inhaled by an EC user. The latter platform was used in conjunction with 3D human bronchial epithelium. A cytotoxic concentration of menthol was determined using the MTT assay in submerged culture. Menthol exposure significantly increased calcium influx and mitochondrial ROS via the TRPM8 receptor. These responses were inhibited by BCTC, a TRPM8 antagonist. VITROCELL cloud chamber exposure of BEAS-2B cell monolayers produced an increase in oxidation of mitochondrial proteins, increased expression of mitochondrial specific antioxidant enzyme SOD2, activation of NFκ-B, and secretion of inflammatory cytokines IL-6 and IL-8. Proteomics data collected following ALI exposure of EpiAirway tissue in the Cultex exposure system was analyzed using Ingenuity Pathway Analysis (IPA) and DAVID software. Menthol exposure of EpiAirway showed upregulation of NRF-2 mediated oxidative stress (z-score = 2.236), oxidative phosphorylation (z-score = 2.828), and IL-8 signaling (z-score = 2.714) as well as a downregulation of cell cycle regulation (z-score = -2.236), PTEN Signaling (z-score = -2.333), and HIPPO Signaling (z-score = -2.449). Our data are in good agreement across the three platforms and show that menthol causes adverse effects on respiratory epithelium via oxidative stress and inflammation, changes that could lead to disease with chronic exposure.

3201 Activation of Sensory Irritant Receptors by Pulegone, a Flavoring Monoterpene in Mint- or Menthol-Flavored Electronic Cigarettes

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Pulegone is a naturally occurring organic monoterpene that is widely present in mint plants, including peppermint, spearmint and pennyroyal. As an ingredient of mint oils derived from these plants, pulegone is often found in food flavorings. Pulegone was recently detected in menthol/mint-flavored e-cigarettes and smokeless tobacco products in amounts that exceeded safety margins, raising health concerns. Pulegone is a group 2B carcinogen with clear evidence for promoting respiratory metaplasia and other neoplasms in rodent oral toxicity studies. Pulegone is also a respiratory irritant, producing strong sensory and epithelial irritation in the nose and lower airways upon inhalation. TRP ion-channels such as TRPA1, TRPV1 and TRPM8 serve as irritant and thermoreceptors in the sensory nerves innervating the airways. They are activated by aldehydes, acids and diverse flavor chemicals and initiate sensory irritant effects or chemesthetic effects such as cool or hot sensations. It is unclear which of these receptors are targeted by pulegone to elicit irritation. Using calcium microfluorimetry of 293T cells transiently expressing human and rodent orthologs TRPA1, TRPM8 & TRPV1 we demonstrate that pulegone has differential and species-specific pharmacological effects on irritant receptors. Pulegone robustly activated human TRPA1 but had very limited effects on rodent TRPA1 orthologs. Pulegone acted as a partial agonist of TRPM8 and TRPV1 for all species tested. Pulegone also inhibited menthol-induced TRPM8 activity, observed for both human and rodent TRPM8. Taken together, the data suggests that pulegone is a strong agonist of human TRPA1 irritant receptors and inhibits the TRPM8 cold/menthol receptor, potentially increasing irritant effects further. These mechanisms may underlie pulegone's strong irritant effects in the nose and lower airways.

3202 Transgenerational Effects of *In Utero* Secondhand Smoke Exposures on Asthma Development in Mice

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While the significant contribution of maternal smoking to the rise of asthma worldwide is well documented, recently, childhood asthma has also been associated with grand-mother smoking, indicating a potential trans-generational heritable effect. However, the mechanisms by which cigarette smoke (CS) or even second-hand smoke (SHS) exposures cause these trans-generational effects on lung disease development are currently unknown. It was previously demonstrated that CS induces a persistent activation of macrophages in the lungs, which produce matrix metalloproteinase-12 (Mmp12). This elastolytic enzyme is involvement in airway inflammation and remodeling. This study aimed to investigate the trans-generational effects of *in utero* SHS exposures on asthmatic responses in wild-type (WT) and Mmp12 knockout (KO) mice offspring. The parental WT and MMP12KO dams were exposed to 10 mg/m^{3}of SHS for 4 hr/day from gestational days 6 to 19. Respective control mice were exposed to filtered-air. Sub-groups of male F1 and F2 generation controls and SHS-exposed offspring were exposed to house dust mite (HDM) via intranasal instillation, to induce asthma-like symptoms; respective controls were treated with saline. The F1 and F2 offspring generations were sacrificed during adulthood. Biological endpoints included broncho-alveolar lavage fluid (BALF) analysis, lung histopathology, and gene expression. BALF analysis showed inflammatory responses to SHS plus HDM exposures. We evaluated the expression of 16 genes involved in chronic respiratory illness and we found that in both generations, 10 genes were up-regulated, including Ccl8, Ccl24, Ear11, Il13, and Muc5ac, and 4 genes were down-regulated, including Ahrr, Igf1, Lmo2, and Nfkb. Overall, these results suggest that *in utero* exposures to SHS may affect the susceptibility to develop asthma later in life and this effect may persist over generations.

3203 A Comparative Analysis of Salivary and Nasal Inflammation Biomarkers in Users of E-cigarettes, Hookah, and Cigarettes

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The effects of cigarette smoking on human health have been extensively investigated, but the impacts of other tobacco derived products, hookah and e-cigarettes, are less known. Saliva collection is the prevailing non-invasive method to detect a range of biomarkers in clinical and research settings. Recent advances in biological sample collection and subsequent analysis

techniques have led to a non-invasive sampling method of the epithelial lining fluid (ELF) from the nasal passage. The ELF method collects a consistent volume, allowing quantification of low-abundance soluble biomarkers such as IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-1ß, INF-y, and TNF-a. These inflammatory biomarkers can indicate the presence of chronic inflammation caused by cigarette, e-cigarette, and hookah use. Several studies have quantified inflammation marker levels in individuals utilizing either saliva or ELF, but no data have been published comparing saliva and ELF samples from the same individual for smokers or vapers. In this study, baseline samples of saliva and ELF were collected from 13 cigarette smokers, 17 e-cigarette vapers, 7 hookah smokers, and 16 non-smokers. Ten inflammation-related cytokines were quantified using the Meso Scale system. Seven of the biomarkers from the ELF of vapers showed significant differences from smokers and non-smokers, while no significant differences were seen in the salivary biomarkers.

3204 An Integrated *In Vitro* Mechanism of Action Assessment Approach for Evaluating E-cigarette Flavoring Compound Toxicity

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The use of flavors is important in the development of electronic nicotine delivery systems (ENDS). However, many flavors do not have safety or toxicity data. In this study, we structurally grouped over 200 common flavors into 38 groups and identified the Flavor Group Representatives (FGR) for each group. We evaluated the biological activity of each FGR and flavor mixture (with and without nicotine) by using a mechanistic *in vitro* screening assays of the real-time cellular analysis (RTCA) and the ToxTracker™ assay. RTCA with primary human bronchial epithelial cells helped identify the most cytotoxic flavors among individual FGRs, including (E,Z)-2,6-nonadienal, alpha-damascone, ambrox, alpha-pinene, 2-methoxy-4-methylphenol, benzyl alcohol, and delta-nonalactone. A parallel *in vitro* test was performed by using the mouse stem cell reporter assay (the ToxTracker® assay) to decipher the potential mechanism of action of each of the FGRs and flavor mixtures in terms of genotoxicity. Flavors and flavor mixtures with a positive signal in the initial ToxTracker® assay were further assessed in human TK6 cells by the *in vitro* FACS-based micronucleus assay, paired with the Multiflow™ assay (DNA-damage signature analysis as well as reactive oxygen species scavenger assay). This study demonstrates that *in vitro* screening assays such as RTCA and DNA-damage signature assays allow mechanistic investigation of the potential contribution of individual flavors to the cytotoxicity and genotoxicity of flavor mixtures.

3205 A Six-Month Inhalation Toxicology Study in ApoE$^{-/-}$ Mice Demonstrates Substantially Reduced Effects of E-vapor Aerosols on Cardiorespiratory Diseases

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Chronic cigarette smoke (CS) exposure causes cardiorespiratory diseases in smokers. Considerable attention has been given to the reduced harm potential of e-vapor products. ApoE$^{-/-}$ mice were used to evaluate lung inflammation, emphysema, atherosclerosis, and cardiovascular function upon chronic (6-month) exposure to fresh air, CS, or e-vapor aerosols. Mice were exposed to CS from 3R4F cigarettes or e-vapor aerosols (generated by using capillary aerosol generators) from various e-liquids (CARRIER: humectants [propylene glycol and glycerin]; BASE: humectants and nicotine; and TEST: humectants, nicotine, and flavors) via the whole-body inhalation system. Exposure to CS (35 µg nicotine/L) caused adverse effects on the lungs (including increased lung volume and inflammation), accelerated atherosclerotic plaque formation, and altered the cardiorespiratory transcriptome. CS exposure caused impairment of systolic and diastolic cardiac function, as assessed by ejection fraction, fractional shortening, isovolumic relaxation time, and E/A ratio. In contrast to CS, e-vapor aerosol exposure did not accelerate atherosclerotic plaque formation, was associated with limited to absent inflammatory cells in the lung tissue, and induced substantially fewer molecular changes in the cardiorespiratory transcriptome. Ultrasound analysis revealed that cardiac dysfunction and aortic stiffness were less prominent in the nicotine-containing e-vapor aerosol-exposure groups than in the CS-exposed group. In conclusion, these results suggest that, relative to CS, e-vapor aerosols induce substantially lower biological responses associated with smoking-related cardiovascular and pulmonary diseases.

3206 Novel Uses of Analytical Platforms for Efficacy/Safety Biomarker Selection to Improve De-Risking of Therapeutics

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Monitoring for drug-induced organ toxicities remains a challenge. A critical gap is the availability of tools to specifically and sensitively detect changes in the emerging variety of biomarkers. The Critical Path Institute's Predictive Safety Testing Consortium, industry, and academic researchers have performed prospective preclinical and clinical studies that reveal the unique challenges and benefits of novel biomarker detection technologies. Best practices were established to ensure reproducible approaches for identifying gene and protein expression profile changes in biopsies, single cells, blood, and/or urine samples that define and reveal cross-species concordances. The most robust platform for analyte measurement depends on the biological matrix and anticipated biomarker context of use. Next-generation multiplex instruments and assays have been developed to support microRNA, gene transcript, and protein quantitation. These tools provide a variety of species-directed high-throughput ribonucleic acid sequencing (RNA-seq) assays and antibodies to discover and validate biomarkers that distinguish injury to specific organs. The aim of this session is to provide an overview of analytical platforms, assays, and translational, drug-induced organ-specific biomarkers to support risk assessment.

3207 Next-Generation Toxicologist Training through Innovative Summer Internships

L. Aleksunes. *Rutgers, The State University of New Jersey, Piscataway, NJ.*

Establishing a pipeline for the next generation of scientists is critical for the advancement and expansion of toxicology. Only a limited number of students are exposed to toxicology through curricula in typical undergraduate science majors. As a result, immersion experiences, including fellowships and internships, provide intensive training of undergraduates in toxicology. Typically, summer programs include full-time mentored research in toxicology for up to three months. These experiences cover responsible conduct of research, experimental design, literature evaluation, data interpretation, and scientific communication. Cohort experiences that engage multiple undergraduate students provide the opportunity for networking and other career-directed activities. This session aims to provide five-minute talks by successful summer program directors and principal investigators from academia, government, and industry that highlight innovations in undergraduate student engagement in toxicology research. A "blitz" of short talks will provide tangible examples that individual scientists and programs can apply to designing and developing their own summer research experiences. This session will be particularly valuable to research advisers, program directors, and near-peer mentors (including graduate students and postdocs). Topics that will be highlighted include innovation in recruitment, resources, weekly programming, peer-peer networking, faculty training, diversity and inclusion, field trips, internships in pharmaceutical companies, and social enrichment. Initiatives include pipelines between liberal arts colleges and research universities, as well as long-term sustainability of programs using sound financial models. Opportunities for funding summer internships from federal sources, such as the National Institutes of Health, and organizations, including the SOT internship program, will be reviewed. The second half of the session will include a moderated panel discussion of various approaches to recruitment, mentoring, team-building, networking, presentation, assessment, matriculation into PhD programs, and long-term tracking. During the panel discussion, attendees are encouraged to ask questions of successful program directors with deep experience in developing and maintaining successful summer internships.

3208 Human Stem Cell-Derived Test Systems as Alternative Approaches for Developmental Neurotoxicity (DNT) Evaluation: Research and Regulatory Perspectives

A. Price. *European Commission Joint Research Centre, Ispra, Italy.*

There is consensus between scientific stakeholders from regulatory agencies, academia, and industry calling for a new framework for regulatory developmental neurotoxicity (DNT) testing based on implementation of alternative approaches relevant to human brain development. A variety of methods have been developed over the past decade to assess neurotoxicity, developmental toxicity, and developmental neurotoxicity, based on human biology instead of animals. Stem cell-based methodologies have taken centerplace here. Although these methods are widely used in research, their implementation within a regulatory context has been slow. This Symposium will give an overview of the human stem cell-based methods for (developmental) neurotoxicity evaluation, and it will highlight their status of regulatory implementation, hurdles encountered on the way there, and strategies to promote their increased use. A pivotal step for regulatory implementation is confidence building, with respect to the reproducibility, reliability, and relevance of the methods. To facilitate this process, methods will be regarded as to their position on a quantitative readiness scale developed two years ago in an international collaboration of regulators and scientists. Requirements for readiness differ for various applications (e.g., for initial screens to prioritize compounds for further testing versus risk assessment of a final product), and this determines the panels of methods currently available for the respective regulatory applications. New developments of stem cell-based neuronal/glial *in vitro* methods will be discussed in light of their use as alternative *in vitro* test systems for (D)NT evaluation for different regulatory purposes (screening and prioritization, hazard identification/characterization, and risk assessment). The ongoing international DNT project under the umbrella of the OECD and in close collaboration with the European Food Safety Authority, US Environmental Protection Agency and US Food and Drug Administration, Health Canada, and Japan that aims to develop the OECD Guidance Document on an *in vitro* battery of DNT assays' regulatory application and data interpretation will be presented. Scientists with relevant expertise in basic stem cell research and regulatory toxicology will present the potentials and limitations of human stem cell-derived neuronal/glial cultures for evaluation of peripheral and central nervous system (D)NT. Two dimensional (2D) models will be characterized against three dimensional (3D) culture systems, discussing the implementation of the *in vitro* battery of assays that permits evaluation of key neurodevelopmental processes. Furthermore, recent studies in which assembly of organotypic vascular and brain tissues has been explored will be presented, with emphasis on reproducibility and data transferability. The difference in response of neuronal culture derived from human pluripotent stem cells to a single chemical and in mixture will be evaluated using *in vitro* assays anchored to key events identified in the adverse outcome pathways network relevant to DNT (AOP-Wiki). The session attendees will have a better understanding of the benefits, challenges, and applications of stem cell-derived *in vitro* models for disease modeling and neurotoxicological studies, with emphasis on DNT evaluation for regulatory purposes. They will be informed on current international efforts that should soon result in the development of the first OECD Guidance Document on an *in vitro* approach to DNT testing.

3209 Differentiation of Human Pluripotent Stem Cells to Specific Neuronal Lineages to Study Windows of Susceptibility and Lineage-Specific Effects of Toxicants: Perfluoralkyl Substances and Methylmercury

A. Bowman. *Purdue University, West Lafayette, IN.*

Human induced pluripotent stem cells (hiPSCs) can be differentiated down specific neuronal lineages in a manner that models typical developmental ontogeny. Such ontogeny-recapitulating methods yield the temporal expression patterns of neural lineage specific markers in sequence to their expression seen during embryonic development. Thus, by timing *in vitro* exposures during hiPSC differentiation, it may be possible to model windows of developmental exposure susceptibility in a lineage specific manner. Further, experimental approaches can be established to determine whether exposures tied to a specific developmental time point will impact subsequent developmental markers and function. We have established methods to differentiate hiPSCs along a cortical glutamatergic lineage and a floorplate dopaminergic lineage. Exposure of both lineages during two key developmental stages: (1) early neuroprogenitor stage, and (2) at the post-mitotic neural specification stage—corresponding to *in vitro* differentiation time points of days 4-10 and 14-20. Continued differentiation and maturation to early premature neurons day 60 and beyond permits assessment of mature neuronal markers and functional characterizations. Exposures to three known or suspected neurodevelopmental toxicants are being examined: methylmercury (MeHg) and two perfluoralkyl substances, perfluorooctanesulfonic acid (PFOS) and perfluorooctanic acid (PFOA). Continuous exposure over one or both developmental windows at concentration curves spanning environmentally relevant exposures (MeHg: 0.1-10 μM; PFOS and PFOA: 4-4,000 ppb) is being performed and comparative toxicological and developmental outcomes measures assessed at early and late developmental time points. These data will be used to highlight the strengths and weaknesses of this approach to understand developmental neurotoxicity.

3210 Robust and Scalable Assembly of Human Neurovascular Tissues for Disease Modeling and Discovery

W. L. Murphy. *Forward BIO Institute and University of Wisconsin–Madison, Madison, WI.* Sponsor: A. Price

The need for human organotypic culture models coupled with the requirements of contemporary drug discovery and toxin screening (i.e., reproducibility, high-throughput, transferability of data, clear mechanisms of action) frame an opportunity for a paradigm shift. The next generation of high-throughput assay formats will require a broadly applicable set of tools for human tissue assembly and analysis. Toward that end, we have recently focused on (1) generating iPS-derived cells that properly represent the diverse phenotypic characteristics of developing or mature human somatic cells; (2) assembling organotypic cell culture systems that are robust and reproducible; (3) translating organotypic cell culture models to microscale systems for high-throughput screening; and (4) combining genomic analyses with bioinformatics to gain insights into organotypic model assembly and the pathways influenced by drugs and toxins. This talk will emphasize assembly of organotypic vascular and brain tissues. These tissues mimic aspects of human organ structure and can be used for reproducible identification of drug candidates and toxicants by both academic and industry scientists. We particularly emphasize reproducibility and data transferability, which are important for the widespread use of organotypic human models in toxicity testing, including in emerging industry applications. The talk will also describe assembled human tissues as models of rare neurodevelopmental disorders of the brain.

3211 Mechanistic Studies of Developmental Neurotoxicity Induced by Exposure to Mixture of Chemicals Using Human *In Vitro* Model for Regulatory Purposes

A. Price. *European Commission Joint Research Centre, Ispra, Italy.*

Key neurodevelopmental processes are vulnerable to perturbations by exposure to environmental chemicals that induce toxicity through multiple mechanisms. We have confirmed in hiPSCs-derived mixed cultures of neuronal and glial cells the physiological response of Nrf2 (nuclear factor erythroid 2-related factor 2) and CREB (cAMP responsive element binding protein) signaling pathway upon specific stimulation and inhibition. In the presence of oxidative stress, Nrf2 nuclear translocation and the upregulation of Nrf2-target enzymes (NQO1, SRXN1, and HMOX1) was observed. The inhibition of the CREB pathway decreased neurite outgrowth and synaptogenesis, presumably through decreased BDNF production. In the follow-up studies, using the same hiPSCs-derived mixed neuronal/glial cultures, the effects induced by mixtures of heterogeneous classes of chemicals (industrial chemicals, drugs, pesticides, endocrine disruptors, and cosmetics) acting through similar and dissimilar modes of action have been evaluated in comparison with a single chemical exposure. The applied assays were anchored to common key events (including oxidative stress and alternations in BDNF levels) of adverse outcome pathways (AOPs in AOP-Wiki), resulting in learning and memory impairment in children. The readiness of these assays for regulatory use was evaluated following semi-quantitative criteria. Based on the obtained results, a battery of *in vitro* DNT assays is proposed for the potential identification of chemicals associated with learning and memory impairment in children, discussing how observed *in vitro* cellular alternations could facilitate mechanistic understanding of behavioral changes. These assays could be included in an Integrated Approaches to Testing and Assessment for different regulatory purposes since learning and memory evaluation is required for DNT regulatory testing.

3212 Stem Cell-Based Developmental Toxicity Testing of the Peripheral Nervous System in Various Regulatory Contexts

M. Leist. *EU-ToxRisk, Konstanz, Germany.*

In the past years, pluripotent human stem cells and their progeny have been used increasingly to establish assays for neurotoxicity testing. Many examples on the measurement of positive controls and underlying mechanisms of toxicity are available, but regulatory uptake has been slow. We present steps to overcome this for tests addressing toxicity to the peripheral nervous system. Three different assays are described here: UKN1 measures early neural differentiation (up to the neuroepithelial stem cell stage), the cMINC measures the capacity of peripheral nervous system precursors (neural crest cells) to migrate, and the PeriTox test measures formation and function of peripheral nervous neurites. Tests were used for regulatory mock submission dossiers to obtain feedback from regulators on the sufficiency of test documentation, validation state, and data relevance. The tests also were used to establish QSAR for potential neurotoxicity of novel cancer drugs and to support read-across hazard evaluation of valproic acid derivatives. The data suggest that the stem cell-based tests for peripheral neurotoxicity are robust and sensitive for questions in a regulatory context. The three tests together detected 48% of a large panel of developmental neurotoxicants. However, many of these toxicants have modes of actions not covered by the test we focused on. Therefore, they will have to be integrated into a larger test battery, which is then expected to have higher sensitivity. A pilot study on this is ongoing, and the outcome is likely to be available by the end of 2019, and to be presented in the session.

3213 2D and 3D Stem Cell-Based *In Vitro* Methods for Assessing DNT: From Basics toward Regulatory Application

E. Fritsche. *IUF—Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany.*

Testing for DNT according to the OECD/US Environmental Protection Agency guidelines is performed in rats. This rodent bioassay does not allow the evaluation of large numbers of chemicals because it is too resource intensive. In addition, species differences between rodents and humans might impede extrapolation of testing results to humans. Due to these obstacles, a DNT *in vitro* testing battery has been assembled that is currently challenged with more than 100 chemicals. Under the umbrella of the OECD and in a collaborative effort with several regulatory agencies as well as academia and industrial partners, a DNT *in vitro* guidance is being developed, taking the future results of the testing battery into consideration. Besides guiding how to perform DNT *in vitro* assays, this OECD guidance will advise on data interpretation, considering metabolism, biological barriers, and temporal aspects of brain development. Also, limitations of the battery (e.g., concerning spatial complexity of brain development) will be pointed out. Results of the testing battery of the test methods neural progenitor cell (NPC)1-5 (i.e., NPC proliferation, migration, differentiation into neurons and glia) will be presented. These methods are thought to predict human DNT; however, due to the lack of human data, only few examples of *in vivo* to *in vitro* extrapolation with regard to toxicodynamics will be given. Toxicokinetics will be covered by *in silico in vitro in vivo* extrapolation (IVIVE). In addition, as the test methods of the battery will be subject to change as science moves on, data on hiPSC-derived 2D and 3D neural differentiation will be shown as examples of test development at an earlier stage. Here, the focus will be on standardization and reproducibility of endpoint measurements. Advantages of fluorescence-labeled target cells will be presented. Results of this current work in progress will reveal suitability of NPC test methods for DNT *in vitro* testing as part of a testing battery. This DNT *in vitro* battery aims to be implemented in regulatory frameworks by the OECD guidance and may finally be included into the OECD DNT TG426 as a basis for more targeted *in vivo* testing. In the long run, it is anticipated to fully replace the *in vivo* TG 426 with a combination of *in silico* and *in vitro* assays. This battery not only will reduce the number of animals used but also will provide a new business opportunity for CROs.

3214 Improving Our Understanding of Toxicant Metabolism and Cytochrome P450s Using Novel Knockout Models and High-Throughput Methods

D. Carlin. *NIEHS, Research Triangle Park, NC.*

The relationship between exposure to environmental chemicals/pharmaceuticals, tissue dose, and toxic mechanism cannot be properly understood without a thorough understanding of compound metabolism, whether it is bioactivation or detoxification, or somewhere in between. Specifically, a thorough understanding of the role that the major metabolic enzyme family, the cytochrome P450s (CYPs), plays in metabolism is needed, as these enzymes are of critical importance in the detoxification of harmful environmental chemicals and drugs. CYP enzymes are particularly challenging to study because they may vary in their metabolism of chemicals, they exhibit significant overlap in substrate specificity between isoforms, and they have large differences in complement and function across species. To add to these challenges, toxicology is moving toward high-throughput assays in toxicity testing due to the expanding number of chemicals found in commerce and the environment. In this session, we will examine the state-of-the-science of CYP in metabolism, novel *in vitro* and *in vivo* high-throughput assays, the challenges to implementation of these assays, and the development of various novel knockout and humanized models to support extrapolation of data from these systems. This session also will highlight future directions for the application of these systems in intervention and prevention of exposure to harmful environmental chemicals and to accelerate the prediction of toxicity of novel pharmaceuticals.

3215 The Future of P450 Research: Basic Questions and Practical Applications

F. Guengerich. *Vanderbilt University Medical Center, Nashville, TN.*

Our collective knowledge about the application of cytochrome P450 (P450, CYP) research to problems in toxicology has grown considerably since the enzyme system was discovered 57 years ago by Omura and Sato. These enzymes are the major catalysts in the biotransformation of drugs, environmental pollutants, and chemical carcinogens, and fundamental knowledge about them has led to important advances in toxicology and pharmacology, as well as fields as diverse as endocrinology, environmental remediation, and biotechnology. As the field has matured, more detailed investigations are in order. Major ones include the molecular basis of the broad specificity of P450s and the nature of the conformational changes that permit P450s to catalyze oxidations of so many molecules. An important deficit is the effects of binding of accessory proteins to P450s on their structures, regarding their functions. Another structural biology deficit involves the genetic variants of human P450s, as related to abnormal function and, in some cases, phenotypic deficiencies related to disease susceptibility. This laboratory has been interested in several of these questions, as well as the continuing issue of how to annotate P450s in regard to their functions (i.e., classifying "orphan" P450s in terms of catalytic function). In addition to these fundamental science problems with P450s, there are many applied issues to address as well. Major questions involve applications to using our knowledge of human variants to avoid drug toxicity and increase efficacy, as well as to better understand roles of these variations in responses to environmental exposures. There also are issues related to genetic variations on an international scale. We have identified oxidative damage in human P450s (sulfenylation, including the heme thiolate) but need to address the significance of this and other oxidative damage under physiological conditions. There are many facets of P450 research that still remain to be addressed.

3216 High-Throughput Screening for Zebrafish Cytochrome P450 Substrates Provides Insight into Enzyme Function

J. Wilson. *McMaster University, Hamilton, ON, Canada.*

The functions of many mammalian cytochrome P450 (CYP) enzymes are well understood, and there are specific and selective probe substrates to assess enzyme expression and function. Yet, there are significant numbers of orphan CYP enzymes, and functional characterization of these proteins is very challenging. These challenges are due to complex evolution and gene duplications in the families responsible for chemical detoxification and co-expression of CYP enzymes in detoxification organs. While heterologous expression of individual CYP enzymes proffers the assessment of individual CYP *in vitro*, deploying individual assays for compound metabolism is a slow and arduous process to characterize function. High-throughput screening offers an exciting opportunity to more quickly provide data on CYP-mediated com-

pound metabolism. This talk will outline the major issues with assessing CYP-mediated compound metabolism, based on evolutionary differences in gene complement and the overlapping capacities of zebrafish enzymes for fluorogenic probe substrates, using CYP1 and CYP3 enzymes as primary examples. To address these issues, we have developed a high-throughput screening approach based on the consumption of the co-factor nicotinamide adenine dinucleotide phosphate (NADPH) as an endpoint to indirectly measure catalytic activity. Using heterologously expressed enzymes and a compound library of pharmacologically active, natural or off-patent small molecule compounds, we have screened zebrafish CYP1 and CYP3 enzymes. The screen and follow-up provide key data to assess the important role of CYPs in xenobiotic metabolism and a direct assessment of the potential for neo- or sub-functionalization of CYP enzymes.

W 3217 Cytochrome P450 Knockout Zebrafish in Toxicology

J. Goldstone. *Woods Hole Oceanographic Institution, Woods Hole, MA.*

Zebrafish are important test organisms for mechanistic toxicological research and for the safety assessment of manufactured and environmental chemicals, yet aspects of metabolism critical to the use of this model are not fully understood. Zebrafish are used in a regulatory context for environmental toxicology in Europe and have become an important toxicology model in the US. However, while cytochrome P450 gene regulation is an important bioindicator in zebrafish, the functioning of the cytochrome P450 systems in xenobiotic metabolism is not well described. Gene-engineered model organisms offer potent systems for studying metabolism and disposition. We have produced and partially characterized transgenic knockout zebrafish for five CYP1 genes, as well as for the orphan CYP20A1 gene. Knockout strains exhibit generally normal development, suggesting that endogenous substrates of these P450s may be generated by other P450s (substrate overlap) or may not be essential for laboratory reared zebrafish. Xenobiotic and indicator substrate metabolism also is altered in these knockout strains. In particular, CYP1A-null zebrafish larvae exhibit strongly decreased whole body ethoxy- and methoxyresorufin (EROD and MROD) metabolism when treated with beta-naphthaflavone for 24 hours, although the EROD/MROD ratio is nearly the same. In addition, the "orphan" CYP20A1-null zebrafish exhibits altered behavior in different behavioral paradigms, particularly those correlating with hyperactivity. Zebrafish increasingly are being used as predictive models of toxicity in various organs, with predictivity in the range of 70%-91%. This research provides a mechanistic understanding of CYP function in a powerful *in vivo* model.

W 3218 Transgenic *Caenorhabditis elegans* Models for Studying P450-Mediated Drug- and Toxicant-Induced Toxicity

J. Hartman. *Duke University, Durham, NC.*

Cytochrome (CYP) P450 enzymes (P450s) metabolize hydrophobic drugs, pollutants, and endogenous chemicals by a monooxygenase reaction that can result in detoxification or, paradoxically, bioactivation to reactive metabolites. Although the metabolism of many chemicals by P450s has been studied in-depth through *in vitro* and *in vivo* experiments, many more chemicals' toxicity and, importantly, mechanism of toxicity are not well understood. As a new tool to study the consequences of P450 metabolism, we developed transgenic *Caenorhabditis elegans* nematodes that express human cytochrome P450 enzymes or orthologs. *C. elegans* have 84 cytochrome P450 genes in their genome; however, total P450 expression levels and activity for common P450 marker substrates are very low relative to high-expression vertebrate tissues such as liver, giving a minimal background in these transgenic animals. The animals are also short-lived (with median life span about 17 days), allowing toxicity outcomes to be measured throughout their lifetime. Other benefits of *C. elegans* include a fully sequenced and well-annotated genome, access to genetic mutants and/or RNAi knockdown of most genes in the genome, ease of generating transgenic mutants through various methods including CRISPR/Cas9, and availability of many fluorescent reporters that allow for medium-throughput analysis of gene expression, redox status, and other measures. So far, we have successfully generated nematodes expressing zebrafish CYP1a and human CYP2E1. We have measured expression of these enzymes at the mRNA and protein level, and enzymatic activity using EROD and 4-nitrophenol assays, respectively. We cannot detect either enzyme expression or activity in wild-type animals but are able to measure robust expression and activity *in trans*genic animals. Compared with wild-type, transgenic CYP1a-expressing nematodes are dramatically protected from PAH toxicity, while CYP2E1-expressing animals are more sensitive to toxicity from the drug acetaminophen. In ongoing and future studies, we are further utilizing CYP1a- and CYP2E1-expressing animals, creating a CYP1B1-expressing nematode, as well as targeting expression of these P450 enzymes specifically in cells of interest, such as dopaminergic neurons. This model holds promise for mechanistic toxicological studies of compounds that are metabolized through cytochrome P450 enzymes.

W 3219 Novel Knockout Animal Models for Studying P450-Mediated Toxicity

W. Baldwin. *Clemson University, Clemson, SC.*

Our laboratory is investigating the roles of CYPs in the regulation of lipid metabolism, obesity, and liver disease in mouse models, and the mechanisms by which toxicants disrupt lipid homeostasis. We recently developed a novel Cyp2b-null mouse model using Crispr/Cas9 that lacks the hepatic Cyp2b members, Cyp2b9, Cyp2b10, and Cyp2b13, found in tandem repeat to test whether loss or inhibition of CYPs increases non-alcoholic fatty liver disease (NAFLD) and obesity. Cyp2b-null male mice develop NAFLD and are diet-induced obese (60% fat; HFD) in comparison with wild-type mice. Interestingly, Cyp2b-null male mice develop steatosis regardless of diet. Thus, normal diet (18% fat)-fed Cyp2b-null male mice show a similar RNA-seq-generated transcriptomic profile as HFD-fed wild-type mice with significant changes in lipid metabolism pathways, including PUFA metabolism and fatty acid elongation. Because of the increase in NAFLD in Cyp2b-null mice, we investigated the effects of a methionine-choline deficient diet on HFD-induced fatty liver disease. This research confirmed that Cyp2b-null male mice are more susceptible to NAFLD and non-alcoholic steatohepatitis (NASH) than wild-type mice. In contrast, female Cyp2b-null mice are less susceptible to NASH than wild-type mice. We also just finished producing a transgenic humanized CYP2B6 (hCYP2B6-Tg) mouse model on the Cyp2b-null background, and CYP2B6 reversed the diet-induced obesity found in Cyp2b-null mice. Targeted lipidomics suggests that changes in linolenic acid metabolism may be responsible for these effects. Lastly, the presentation also will briefly discuss the phenotype of Cyp3a-null mice as in contrast to Cyp2b-null mice, the females are somewhat resistant to diet-induced obesity. Overall, the purpose of this project is to test whether alterations in Cyp2b and Cyp3a activity (such as chemical inhibition) can alter lipid metabolism and in turn cause NAFLD and obesity.

W 3220 New Frontiers in Dynamic Toxicology

J. Wambaugh. *US EPA, Research Triangle Park, NC.*

The evolution of toxicity over time has often been characterized with serial observations and models based upon differential equations. New techniques are becoming available that allow additional insight into the dynamics of toxicology from various perspectives. In addition to addressing when, the speakers in this session describe tools for understanding who, what, where, how, and/or why chemical toxicity and exposure may occur to specific, susceptible populations. Depending upon the mechanism of action, windows of susceptibility exist as part of biological development and aging during which the effect of toxic perturbations may be greatly increased. The timing and magnitude of chemical exposures add a layer of complexity on top of biology, as exposures are subject to human activities and whims. Passive samplers can provide a record of exposure to a variety of chemicals over a time interval of activity. Alternatively, exposomics projects can provide snapshots of diverse biometric and chemical exposure information. Mathematical modeling and dynamic machine learning can allow inferences to be drawn from both time-integrated and instantaneous information. Affect modeling can help address the why of human behavior—what drives patterns of product use and chemical co-exposure? Meanwhile, machine-learning methods for time-series data can draw powerful inferences about what goes on between the sampling intervals. All the new approach methodologies (NAMs) presented can inform modern toxicological assessment of the chemical effects on public health. The Workshop will conclude with a moderated panel discussion where speakers will address audience questions on how to apply the methods presented to toxicology. In this session, each speaker will (1) provide example systems, made relevant to toxicologists, in which important aspects change over time; (2) describe the key challenges in understanding the example systems; (3) characterize which aspects change over time and which are constant; (4) describe publicly available tools or methods for analysis of their examples; and (5) consider how better characterization of dynamics can inform toxicology and chemical risk assessment.

3221 Windows of Susceptibility

A. Lumen. *US FDA/NCTR, Jefferson, AR.*

Several physiological changes occur during gestation to meet the needs of both the mother and the developing fetus. Such increased physiological demands and strained compensatory functions leave pregnant women and the fetus more vulnerable to toxicological perturbations. In this talk, the speaker will introduce the audience to the concept of "windows of susceptibility," specifically focusing on the gestational period. Often, it is implausible to obtain rich toxicodynamic data in these sensitive life stages. Therefore, the need and utility of computational tools such as physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) modeling to make appropriate toxicological evaluations will be discussed. The talk also will feature a case study demonstrating the application of modeling techniques to evaluate perturbations due to environmental contaminant exposure in sensitive gestational life stages (pregnant women and developing fetus). References for published and publicly available algorithms for such computational tools for independent evaluation by the audience will be provided.

3222 Time-Integrated Exposures to Identify Chemical Profiles between Healthy and Dysphagic Foals

B. Rivera. *Oregon State University, Corvallis, OR.*

Passive samplers serve as a sensitive tool to capture time-integrated exposure of an individual. Sequential sampling can be done to determine if exposure profiles vary over time, based on other environmental factors (seasonal variability, location, etc.). In this study, foals born at a location in proximity to unconventional drilling had a higher incidence of dysphagia compared with foals, bred by the same owner, in an area without active drilling. Consecutive sampling was conducted over the entire gestational period of these foals to investigate if an association between exposure profiles and health outcome exists. Halter samplers, capturing individual exposure, and stationary well water and air samplers were used to identify potential routes of exposure associated with unconventional drilling. Analysis of well water samplers identified differences in polycyclic aromatic hydrocarbon (PAH) profiles between the two locations. PAHs with three or more rings were higher in the area with active drilling, and two-ring PAHs were higher in the area not active in drilling. After the installation of a water treatment system at the active drilling location, a decrease in concentration of PAHs with three or more rings was reported. Additionally, during this time, a decrease in incidence of dysphagia also occurred.

3223 Air Force Surgeon General's Total Exposure Health Initiative: At the Intersection of Toxicology and Exposomics

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Health care in the 21st century has been transformed with the availability of a reference human genome sequence accompanied by intensive efforts to understand the genetic basis of disease and treatment on an individualized level as exemplified by President Obama's Precision Medicine Initiative. A strategic vision of the Air Force Medical Service is to craft the "healthiest and highest performing segment of the US by 2025." In a step toward realizing this strategic vision, the Air Force Surgeon General launched the Total Exposure Health (TEH) initiative. TEH envisions transforming health care from a reactive stance to a proactive stance by harnessing informatics to integrate all available data (genomic, lifestyle, occupational, environmental exposures, etc.) to inform the warfighter and commanders of their health risks, fostering health-engendering behaviors and actions. This presentation will address how the Air Force is leveraging and advancing exposure science coupled with employing a systems biology approach that uses toxicological new approach methodologies (NAMs) to include computational modeling and cell biology with proteomics, genomics, metabolomics, epigenetics, and bioinformatics to address TEH requirements in hopes of elucidating underlying mechanisms of effects based on given sets of exposures from a molecular- to human-level perspective. A case study on research in the area of characterizing physiological and environmental effects in the very dynamic environment of high-performance aircraft (almost all parameters change over time: exposure, breathing rate, chemical profile, altitude, humidity, physiological support, etc.) pertaining to in-flight physiological stressors will be discussed, as well as how the Air Force routinely leverages public databases and software to include ECHA, nite, httk, OECD QSAR Toolbox, and ochem.eu to deliver rapid responses to our operational community. Using a systems biology approach to address TEH requirements has great potential to determine/deliver personalized safe exposure levels and knowledge supporting the development of individualized treatment and mitigation strategies.

3224 The Habits and Practices of Beauty: The Technical Truth Underlying Consumer Perception

K. Krishan. *Procter & Gamble, Singapore, Singapore.* Sponsor: J. Wambaugh

Product innovation for fast-moving consumer goods is designed around consumer behavior and linking consumer perception to product performance. The challenge is to deliver delighting experiences across a spectrum of end-use conditions. Physical, biological, social, and environmental conditions lead to usage habits and practices impacting product performance that are hard to reproduce in the laboratory. The forefront of innovation in the beauty industry relies on deeper behavioral insights that determine the short- and long-term benefits of products. This will be illustrated with examples from across this industry. The characterizations here place the context under which toxicological impact factors need to be assessed.

3225 Modeling, Machine Learning, and Time-Series Data

A. Pitruzzello. *Northrop Grumman Corporation, Morrisville, NC.* Sponsor: J. Wambaugh

Wristbands can give us a time-integrated measure of chemical exposure, while exposomics can give us a snapshot of instantaneous exposure and health metrics. How can we bridge the gap? Given a mathematical model characterizing the dynamics of a system, time-series machine learning can allow classification and anomaly detection. These techniques have been applied in systems ranging from medical (ECG signals) to interpreting data from diverse sensor arrays. The speaker will provide commentary on how these technologies can inform inference of cellular dynamics. Brief examples will be given to introduce toxicologists to these powerful, open-source algorithms and tools.

3226 Parental Cannabis Exposure and Long-Term Neurobehavioral Deficits in Offspring

K. Ryan. *NIEHS/NTP, Research Triangle Park, NC.*

Recreational and medical marijuana are not recommended for use during pregnancy. However, cannabis use by pregnant women in the US has increased as much as 62% (2002-2014) with an overall prevalence of use between 3-16%. Current human and experimental evidence suggest that maternal cannabis use during pregnancy is associated with a spectrum of adverse neurological deficits in children or offspring. Similarly, paternal cannabis use causes abnormal epigenetic imprinting on sperm which could impact development of the next generation. However, some discrepancies exist in the available data which could contribute to some confusion among consumers or a lack of concern related to cannabis use during times of fetal development. In addition, many consumers may not be aware of the harmful consequences of cannabis use, by either parent, on fetal development as well as long-term effects on neurocognition and behavior. The firs presentation will highlight the increasing use of cannabis among women during pregnancy and nursing for pain and nausea as well as among men prior to conception. Some misconceptions regarding cannabis use and risk among consumers will also be presented. As a result of increased use, longitudinal studies are linking fetal cannabis exposure to decreased growth, cognitive impairment, and behavior deficits in children. The second presentation will provide evidence suggesting that studies performed in rodents mirror clinical findings and allow more in-depth assessments of neurobehavior and associated molecular phenotypes. Results of various human and animal studies will also be discussed in the context of generating education strategies or interventions to improve the mental health outcomes of children subjected to cannabis exposure *in utero*. In addition to maternal exposure, the third presentation will highlight new evidence suggesting that paternal exposure to cannabis prior to conception can also impact neurobehavior using preclinical rodent models. A goal of this talk is to link cannabis exposure, changes in DNA methylation, and subsequent cognitive impairment as well as bring awareness to this understudied

area of research. The fourth presentation strengthens the weight of evidence by linking parental cannabis exposure and adverse effects on neurodevelopment in a third species (i.e. zebrafish). Use of this complementary model system allows for the assessment of cannabis or its hundreds of components on development, behavior, and reproduction across multiple generations; which is often a resource intensive task in rodent models or human studies. The final presentation will provide insight from the public health perspective. This presentation focuses on the real-world application and utilization of preclinical and clinical research which is critical to the development of public health recommendations and communication of risks related to prenatal cannabis use and potential adverse childhood health outcomes. The workshop will end with an informal deliberation among panel speakers and audience to 1) review the current weight of evidence for neurological deficits in children as a result of parental cannabis use during pregnancy, 2) propose strategies for research data gaps, and 3) discuss communication strategies to highlight risks for consumers. This session brings together experts across clinical and preclinical research settings, including several non-SOT members with expertise specifically identified to highlight the state of research regarding parental cannabis exposure and adverse consequences to the developing nervous system. Importantly, adverse effects on neurobehavior are supported by results across multiple species including humans, rodent models, and zebrafish which emphasizes the need for more toxicological research during critical stages of development. Furthermore, the toxicological science of drugs of abuse has been greatly under-represented at SOT relative to its societal importance and progress in the field. In 2019, SOT had one workshop about the toxicology of drug abuse. That workshop focused on how many types of drug abuse affect adolescence. In 2020, we have a chance to continue our efforts promoting research into the socially important areas of the toxicology of drug abuse, this time with a focused discussion of cannabis exposure during early development and its persisting neurotoxic effects.

W 3227 Marijuana Use in Pregnancy: Sorting through Hazy Evidence

T. Metz. *University of Utah Health, Salt Lake City, UT.* Sponsor: K. Ryan

The prevalence and perceived safety of marijuana use in pregnancy are increasing with expanding legalization in the United States. Marijuana crosses the placenta and passes into breast milk. Some women cite reasons for marijuana use in pregnancy and while breastfeeding, such as nausea, pain, and anxiety. The existing literature is heterogeneous with regard to the impact of marijuana use on pregnancy and early childhood outcomes. A National Academies of Sciences report on the health effects of cannabis concluded that marijuana use is associated with poor fetal growth but that evidence for the association between marijuana use and other perinatal outcomes is inconclusive. There are limitations of the existing literature that preclude firm conclusions as to the effects of prenatal marijuana use. These limitations include a high reliance on self-report for ascertainment of use and incomplete adjustment for confounding factors such as tobacco use. Two recent systematic reviews and meta-analyses found an association between marijuana use and adverse perinatal outcomes, especially with heavy marijuana use. In addition, there are three longitudinal studies examining the relationship between prenatal marijuana exposure and childhood neurodevelopment. While early childhood neurodevelopmental outcomes are similar, investigation later in life demonstrates decreased attention, verbal reasoning, and cognitive function. With the high prevalence of marijuana use among reproductive-age women, it is critical for health care providers to query women regarding use and provide information regarding the potential harms. Given the available evidence, women should be advised to refrain from marijuana use during pregnancy and while breastfeeding.

W 3228 Developmental Consequences of Early-Life Cannabis Exposure

Y. Hurd. *Icahn School of Medicine at Mount Sinai, New York, NY.* Sponsor: K. Ryan

The expanding legalization of recreational and medical cannabis as well as the changing attitudes regarding the harm of cannabis have contributed to a significant increase in women using cannabis while pregnant. Additionally, there is increased prevalence of cannabis consumption among breastfeeding women and of children with secondhand cannabis exposure. Delta-9-tetrahydrocannabinol (THC), the psychoactive component of cannabis, readily crosses the placenta barrier and also is transferred through breast milk, which raises significant concern about the impact of early-life cannabis exposure during these critical periods of development. The goal of our project is to examine the trajectory of behavioral and neurobiological disturbances associated with early-life cannabis/THC exposure. Preclinical rodent models, postmortem human fetal brain specimens, and longitudinal human investigations were investigated in relation to early-life cannabis/THC exposure. Biochemical, molecular, epigenetic, and behavioral studies were carried out at different developmental periods. Findings from our human studies and animal models, in combination with results from the field, emphasize neurobehavioral, socioemotional, physiological, molecular, and epigenetic consequences of developmental cannabinoid exposure that extends from the neonatal period through adolescence and into adulthood. For example, daily maternal exposure to THC (resulting in 10 ng/mL plasma concentration; ~1 joint/day) during pregnancy alters molecular mechanisms related to synaptic plasticity well into adulthood of their offspring, linked to disturbances of histone methylation epigenetic marks and depression-like behavior. The stress system appears to be a particularly critical biological substrate affected by the developmental effects of cannabis/THC. The accumulating data emphasize long-term impact on specific neural systems and phenotypes predictive of psychiatric and addiction vulnerability that altogether provide scientific evidence to help guide interventions and education strategies to improve the mental health outcomes of children and adults.

W 3229 Pre-conception Paternal THC Exposure of Male Rats Causes Long-Term Neurobehavioral Effects in the Next Generation

E. Levin. *Duke University, Durham, NC.*

Maternal exposure during gestation to a wide variety of toxicants has been widely shown to cause neurobehavioral toxicity in the next generation. However, the impacts of paternal toxicant exposure pre-conception on offspring neurobehavioral function have been much less studied. We have found that pre-conception delta-9-tetrohydrocannabinol (THC) exposure in male rats significantly alters sperm DNA methylation and that cannabis smoking in human males is significantly associated with altered sperm DNA methylation in similar pathways (Murphy et al., *Epigenetics*, 13:1208-1221, 2018). The functional impact of THC-induced altered sperm DNA methylation on the next generation is still largely unknown. In the current studies, we investigated the effects of pre-conception THC exposure of young adult male rats (0 or 2 mg/kg/day for 12 days or 0, 2, or 4 mg/kg/day for 28 days) on the neurobehavioral development of their offspring. These dose regimens of paternal THC exposure were not found to significantly impact the clinical health of the fathers or offspring. However, they did cause a long-lasting, significant effects on locomotor activity and cognitive function in the offspring relative to controls when they were tested in adolescence and adulthood. The shorter paternal THC exposure caused a significant increase in habituation of locomotor activity and attentional impairment in the adult offspring of the males exposed to THC prior to mating. The longer paternal THC exposure caused significant locomotor hyperactivity in the adolescent offspring, significantly quicker habituation in novel object exploration, and significantly delayed acquisition of the radial-arm maze in adult offspring. These studies demonstrate that pre-conception paternal THC exposure, even at a modest dose, can cause deleterious neurobehavioral effects in their offspring, including cognitive impairment. Additional research is needed to determine the degree to which this type of neurotoxic risk in offspring is seen in humans, to investigate the mechanisms underlying these effects, and to develop therapeutic treatments to ameliorate these long-term adverse behavioral consequences of paternal pre-conception THC exposure.

W 3230 Assessing Effects in Zebrafish of Parental Exposure to Delta-9-Tetrahydrocannabinol on Long-Term Neurobehavior and Aging Phenotypes

K. Willett. *University of Mississippi, University, MS.*

Countries are relaxing laws regarding the use of cannabinoids such as delta-9-tetrahydrocannabinol (THC). As a consequence of their increased consumption, understanding potential adverse outcomes following exposure to cannabinoids during critical developmental periods such as pregnancy is important. Zebrafish (*Danio rerio*) as a research organism provide a relatively high-throughput model by which both the efficacy and potential toxicity of THC can be assessed. Furthermore, they can be used to determine molecular/cellular mechanisms of the developmental neurotoxicity of cannabis and to more quickly vet the hundreds of different compounds in cannabis products. To assess the acute developmental consequences of THC, zebrafish were exposed through the larval stage to 0.08 to 1.25 μM THC. Developmental deformities, behavioral analysis, and RNA-seq analysis of the transcriptome are being used to identify potential target organ and toxicity pathways. This study investigated developmental origins of health and disease (DOHaD) via multigenerational gene expression patterns, 96 hpf behavior of larval offspring (F1 and F2), locomotive and anxiolytic behavior of adult fish assessed

through open field testing at 12 months, and reproductive fitness of a subsequent F1 following the F0 developmental exposure of zebrafish. Three years following the initial larval exposure, aged F0 fish were assessed to determine if early-life exposure to cannabinoids alters aged fish behavior or offspring survival, as well as aging phenotypes such as brain and liver expression of genes involved in: neurodevelopment (bdnf), proliferation (p53), cell cycle arrest (p16, p21), and immune markers (tnfa, Il-6, Il-1b). Results from this work highlight that zebrafish (1) offer a good tool to measure multigenerational impact of THC exposure, (2) demonstrate that THC effects on neurobehavior mirror effects in rodent models with the advantage of greater throughput, and (3) can inform policy considerations related to THC expanded use.

3231 The Public Health Perspective on Prenatal Cannabis Use: What Do the Data Tell Us, and What Are the Data Gaps?

E. Contreras. *Colorado Department of Public Health and Environment, Denver, CO.* Sponsor: K. Ryan

From the public health perspective, more data are needed to better understand and communicate risks regarding the impact of marijuana use on pregnancy and childhood outcomes such as neurodevelopmental disorders. At the Colorado Department of Public Health and Environment, the Marijuana Health Monitoring and Research program (MHMRP) has an obligation to "monitor changes in drug use patterns and the emerging science and medical information relevant to the health effects associated with marijuana use." This goal is achieved through a panel of health care professionals with expertise in varying aspects of cannabis. Experts are tasked with collecting and reviewing relevant data in the peer-reviewed literature to develop and approve public health recommendations related to cannabis use in Colorado. As a first step, a broad search (e.g., Medline) of current peer-reviewed publications is conducted quarterly, and these are rated as a high-, medium-, or low-quality finding based on the strengths and limitations of the methods via the GRADE system. Next, draft evidence statements for population, exposure, and outcome (health effect) are summarized based on the quantity and quality of evidence. This information is then used to draft public health recommendations (e.g., for education or monitoring) as well as data gaps that are reviewed and approved by the expert panel. Our work has led to the development of 26 evidence statements on marijuana use and pregnancy and breastfeeding. Areas with moderate evidence of concern for maternal use of marijuana during pregnancy include reduced cognitive function, lower IQ, and attention problems in exposed offspring. Unfortunately, most of the evidence for health outcomes such as birth defects, motor deficits, and mental health issues is still limited or mixed. Due to the Schedule I status of marijuana, federally, it is extremely difficult to study any health outcomes related to marijuana. As such, the marijuana industry is outpacing public health regulations due to lags in high-quality research on the health impacts of marijuana use. Overall, it remains imperative that research continue on marijuana use and health impacts, including childhood neurodevelopmental disorders. More research is needed in vulnerable populations like prenatal exposures, second- and third-hand exposures in children, youth use, medical use among immunocompromised populations, and use in populations with mental illness. Additionally, research is needed on how different modes of marijuana use affect health or how potency may or may not exacerbate a health condition. Until all these areas are well described in literature, we will not know the true impacts of marijuana legalization on the health of populations.

3232 Differentiating Respirable Sensitizer and Irritants Using *In Vitro* Models: Analysis of Cell-Based Monolayers versus Co-cultures

M. J. Gibb, and C. M. Sayes. *Baylor University, Waco, TX.*

In an effort to decrease the use of animals used in toxicological research testing, investigations that lead to verifiable *in vitro* testing methods are desperately needed. Unlike that of the skin, the lungs lack validated and universally-accepted *in vitro* testing methods that differentiate between irritating and sensitizing aerosols. To complicate the issue further, these toxicity-inducing aerosols could exist as either liquid droplets or solid particulates. Therefore, validating an *in vitro* model requires both comprehensive aerosol and cell characterization. Previously, we have developed and tested an *in situ* real-time model system that collects both physical, chemical, and biological data before, during, and after aerosol exposure to lung cell cultures at the air-liquid interface. In this study, further investigate *in vitro* based testing systems by utilizing the liquid-liquid interface to compare the irritation versus sensitization effects to cell monolayers and co-cultures of pulmonary cells. The monolayer includes human lung epithelial cells, only; while the co-culture consists of epithelial cells plus human monocytes transformed into macrophage-like phenotype and dendritic cells that function as antigen presenting cells. Results show that after exposure to the known solid particulate irritant crystalline silica nanoparticles, the co-culture model produced significantly more TNF-alpha as compared to the monolayer, suggesting the importance of using multiple cell-types when validating *in vitro* models. Similar results were found when exposing the known sensitizer nickel oxide and subsequently measuring endpoint IL-13. Interestingly, particle silver colloids did not induce a measurable increase in either biomarker (when compared to untreated control cell populations). These results show that *in vitro* co-culture models may be suitable for initial screening assessments of new materials suspected as either irritants or sensitizers. Implementation of this model would aid in the acceleration of bringing newly developed products (and formulations) in the laboratory to the commercial market.

3233 How Sex-Specific Arsenic Immunomodulation Influences Infectious Disease and Cancer Risk

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Inorganic arsenic (iAs) is a potent carcinogen and immunotoxicant, posing a risk to more than 200 million people worldwide through the consumption of contaminated water and food. iAs exposure has been correlated with, often sex-dependent, increases in risks of cancer and respiratory tract infections, including tuberculosis (TB) and influenza. However, it is unclear what the causal mechanisms are. We hypothesize that iAs causes a sex-specific immune imbalance leading to on the one hand increased infectious disease risk, while on the other hand contributing to a tumor-promoting microenvironment. We focused on the effects of iAs (sodium meta-arsenite) on macrophages, innate immune cells known to influence both cancer and TB pathogenesis. We observed sex- and dose-specific suppression of nitric oxide (NO) and cytokine production, phagocytosis and differences in cell surface expression as assessed by flow cytometry in activated bone marrow-derived macrophages (BMDMs) *in vitro* treated with iAs (0.0001 - 1 μM) either during or after macrophage differentiation. Moreover, signaling lipid analysis revealed a skewing towards a tumor-promoting phenotype. We observed similar findings in activated lung macrophages from mice either *in utero* or chronically exposed to 100 ppb and 1000 ppb iAs. We are currently investigating how these macrophage alterations influence TB and cancer risk. Using an influenza A virus (IAV) mouse model, we also studied the sex-specific effects of iAs exposure on the immune responses against primary infection. We observed exacerbated disease severity, proinflammatory cytokine responses and mortality in iAs-exposed females compared to males upon primary IAV infection. Despite an increased early cytokine storm, influenza viral titers remained high in iAs-exposed females compared to controls and males over the course of infection. We are currently investigating how observed differences in immune cell infiltration and function may contribute to the arsenic-induced disease phenotype. Overall, our data elucidate the different ways that iAs influences both cancer and infectious disease risk in exposed populations through immunomodulation. *NIEHS R00ES024808 (FS), T32ES07141 (KR, EI) and 5T32HL007534-37 (SA)*

3234 Development of a T Cell-Specific Nrf2-Null Mouse Model to Determine the Role of Nrf2 in Impaired T Cell Response to Influenza by tBHQ

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Influenza infections cause hundreds of thousands of hospitalizations in the United States each year. Despite increased vaccination compliance, the number and severity of infections have not improved, suggesting there is a disconnect between vaccination and disease protection. Accordingly, there has been considerable interest in identifying factors that contribute to susceptibility to influenza virus infections and/or reduce vaccine efficacy. $CD8^+$ T cells play an indispensable role in clearing virus-infected cells during infection and controlling viral replication by secreting antiviral cytokines. Early work from our lab identified an immunosuppressive effect of the Nrf2-activating food additive *tert*-butylhydroquinone (tBHQ) on murine $CD8^+$ T cell activation *ex vivo*. Accordingly, we hypothesized that tBHQ would suppress the $CD8^+$ T cell response to influenza infection. To test this, wildtype mice were fed diets with or without 0.0014% tBHQ - a dose that correlates well with estimated human exposure and is also the concentration in common rodent chows. Two weeks after acclimation to the diets, mice were challenged with a sublethal titer of influenza A/PR/8/34 (H1N1). At the peak of the T cell response, the $CD8^+$ T cell response to infection was assessed through RNA, ELISA, and FACS analyses.

Mice on the tBHQ-supplemented diet had a reduced number of $CD8^+$ T cells in the lungs and a reduced number of influenza-specific $CD8^+$ T cells in draining lymph nodes compared to their control-diet counterparts. Furthermore, tBHQ reduced $CD8^+$ T cell activation as evidenced by a decrease in the number of $CD25^+$ $CD69^+$ $CD8^+$ T cells and effector function as determined by reduced expression of FasL and CD107a. Moreover, mice on the tBHQ diet had a trend towards increased viral burden in the lungs. To investigate the role of Nrf2 in these effects, Nrf2-floxed mice were crossed with mice expressing Cre under the CD4 promoter to generate mice lacking Nrf2 in both $CD4^+$ and $CD8^+$ T cells. *Ex vivo* analysis of the T cells from these mice demonstrated that Nrf2 expression is ablated and Nrf2 target gene expression substantially decreased, indicating this will be a useful model to assess the role of Nrf2 in T cells during influenza infection. *This work was supported by NIH grants ES024966 and GM092715.*

3235 Cannabidiol-Regulated microRNAs Promote Mast Cell Signaling via Peroxisome Proliferator-Activated Receptor-γ to Protect against Staphylococcal Enterotoxin B-Induced Liver Injury

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Recreational use of cannabis and its components, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), is now legal in several states. Despite the increased popularity of these products, there is a gap in knowledge surrounding the effects of their daily usage. Our previous studies demonstrate that CBD treatment of naïve mice causes significant expansion of $CD11b^+Gr\text{-}1^+$ myeloid derived suppressor cells (MDSC), as a result of increased peroxisome proliferator-activated receptor gamma (PPARγ) signaling. Using a staphylococcal enterotoxin B (SEB)-induced liver injury model, we examined CBD regulation of mast cells during acute inflammation. Herein we report that CBD treatment before SEB exposure prevents immune cell-mediated liver damage. Briefly, mice were intraperitoneal (I.P.) injected with 20mg/kg or 50mg/kg of CBD for 4 days (d), followed by SEB challenge on d 3. Flow cytometry analysis of the peritoneal and liver contents revealed a significant reduction in inflammatory cell types, including T helper, T cytotoxic, and $CD19^+$ B cells. Additionally, we show that despite decreased numbers of $CD11b^+$ $F4/80^+$ macrophages, there was an increase in the total number of $CD11b^+$ myeloid cells. Further analysis revealed that the expanded population of myeloid cells was comprised of $Ly6C^{HI}MHCII^{LO}$ MDSC. Employing microarray analysis, we examined differences in microRNA (miRNA) expression of liver mononuclear cells to identify possible gene targets of CBD. Compared to leukocytes isolated from disease mice, SEB mice treated with CBD displayed enrichment of miRNAs targeting mast cell growth factor, KIT ligand (KITLG). Furthermore, miR-217-5p, which is predicted to interact with the co-activator of PPARγ (PPARγC1A), was significantly downregulated in these mice. Downregulation of miR-217-5p tightly correlated with increases in PPARγ mRNA expression. These findings and our previous data taken together suggest that CBD dynamically regulates miRNAs targeting KITLG and PPARγC1A to enhance mast cell signaling via PPARγ during acute inflammation. Enhanced PPARγ signaling likely induced proliferation of $Ly6C^{HI}MHCII^{LO}$ MDSCs, as it has been shown to result in the secretion of myeloid colony-stimulating factors. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788, and R01AI123947.*

3236 Mechanisms of Cannabidiol-Induced Immunosuppression Mediated by Myeloid-Derived Suppressor Cells

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Myeloid-derived suppressor cells (MDSC) are heterogeneous immune cells that are immunosuppressive against other leukocytes such as T cells. While the immunosuppressive functions of MDSC can contribute to amelioration of inflammatory and autoimmune diseases, high MDSC accumulation in cancer can promote malignancy progression by inhibiting anti-tumor immune responses and interfering with immunotherapy. Cannabidiol (CBD) is a major nonpsychoactive phytocannabinoid derived from cannabis, which has anti-inflammatory properties, and whose use is becoming widespread worldwide. Research from our laboratory has shown that CBD is a potent inducer of MDSCs with robust immunosuppressive activity. In this study, we investigated the immunosuppressive phenotype of CBD-induced MDSCs. Naïve C57BL6 mice were administered CBD (20mg/kg, i.p.) or Vehicle (Veh) and euthanized after 24h. Gr1+ cells were then isolated from bone marrow and peritoneal cavities and subjected to transcriptome expression analysis. Consistent with previous reports from our lab, CBD induced massive expansion of Gr1+ cells in the peritonea, but not in the bone marrow of mice. Identification of the top significantly altered genes revealed that bone marrow compared to peritoneal Gr1+ cells had elevated expression of neutrophil-specific genes such as *Ngp (Neutrophil granulocyte protein), Camp (Cathelicidin antimicrobial peptide),* and *Elane (Elastase, neutrophil-expressed),* confirming that bone marrow Gr1+ cells were predominantly neutrophils, while peritoneal Gr1+ cells were predominantly MDSCs. Subsequent comparison of peritoneal Gr1+ MDSCs between Veh- and CBD-treated mice showed that CBD-induced MDSCs had dramatically elevated expression of *Crip1* (Fold change: +20.77, p=0.0016) and *Arg1* (Fold change: +6.24, p<0.0001), which encode Cysteine-rich intestinal protein 1 and Arginase-1, respectively. Cysteine and arginine are well-known to be necessary for T cell activation. Therefore, our results demonstrate that the potent immunosuppressive functions of CBD-induced MDSCs likely involve sequestration of cysteine and arginine due to upregulation of these genes. These results suggest that CBD-induced MDSCs may have pleiotropic effects on cancer models by dampening anti-tumor immunity or attenuating inflammation-induced cancer. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.*

3237 The Herbicide Glyphosate Directly Affects Human T Cell Responses

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Glyphosate [N-(phosphonomethyl)glycine] is an active ingredient of one of the most used herbicides. Glyphosate targets the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS); a key enzyme of the shikimate pathway which plays a pivotal role in the biosynthesis of aromatic amino acids (AAs). This pathway is present in several organisms, like plants, algae, fungi and bacteria, but neither in animals nor humans. The use of glyphosate is currently a very debated topic as several studies indicating its hazard and toxicity are emerging. There are few human epidemiology studies investigating the correlation with glyphosate exposure and human diseases. But there are several evidences of adverse effects in laboratory animals (i.e. liver, cardiovascular and reproductive toxicity, genotoxicity). In addition, studies conducted on animals suggested that glyphosate could also act as an immunomodulator. In a mouse model, glyphosate was able to promote Th2 type cytokines. This result has been partially confirmed in a previous study we conducted on 26 farmers of Northern Italy professionally exposed to glyphosate. Serum samples presented higher level of IL-4, IL-5, IFN-γ and lower levels of IL-8 and IL-33 upon glyphosate exposure. Lymphocyte subpopulation analysis revealed a higher count of Th1 and Th2 cells and a lower count of Th17, suggesting an immunomodulatory effect of glyphosate characterized by an impairment of the Th1-Th2 profile. Based on these data, the aim of this work was to investigate if glyphosate could directly affect T cells. *In vitro* studies were conducted using peripheral blood mononuclear cells obtained from buffy coats of male donors (n=5). Lympho-monocytes were treated with increasing concentration of glyphosate (0.01-0.1-1-10 μg/ml) in the presence or absence of T cell mitogens, namely PHA and PMA+ionomycin for different times (24-72 h). In agreement with what was observed *in vivo*, *in vitro* treatment resulted in an enhancement in IL-4 as well as IFN-γ levels. Furthermore, miRNA production contained in exosomes was also analyzed as possible mediator of the observed effects. Following *in vitro* exposure to glyphosate an up-regulation of several miRNAs involved in the immune system has been found (i.e. miR-10b and let-7f), consistent with the *in vivo* findings. These results indicate a direct action of glyphosate on immune cells. Further studies are underway to better understand the mechanism of action underlying the direct effects of glyphosate on T cells, while prospective studies will be necessary to understand the prognostic significance of the observed effects.

3238 Aryl Hydrocarbon Receptor Regulation of T Follicular Helper Cells

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Environmental factors influence the function of the immune system in ways that enhance or limit disease. One means by which environmental signals regulate immune responses is via a ligand-activated transcription factor called the aryl hydrocarbon receptor (AhR). The AhR binds structurally diverse molecules, including certain pollutants, dietary factors, and chemicals from microorganisms. AhR activation modulates adaptive immunity, but the cellular mechanisms are not fully defined. Changes in T cells and T cell dependent immune responses are among the most consistently observed effects of AhR activation. For instance, AhR ligands alter the differentiation and function of Th1, Th2, Th17 and Treg CD4+ T cell subsets. AhR activation also affects antibody responses to immune challenges; a process for which another CD4+ T cell subset, called T follicular helper cells (Tfh cells) are critical. Thus, we

hypothesize that the AhR regulates Tfh cell differentiation and function. We show that AhR activation with the prototype AhR agonist, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) reduces the percentage and number of Tfh cells in mice infected with influenza A virus (IAV); changes that correlate with diminished levels of virus-specific, isotype switched antibodies. Generating Tfh cells is a multistep process that involves signaling within CD4+ T cells and from accessory cells. Using mice that lack *AhR* in the hematopoietic compartment, we show that changes to Tfh cells requires AhR expression in immune cells. AhR activation also skews Tfh cell differentiation *in vitro* further supporting that it controls cell autonomous pathways in CD4+ T cells. The AhR regulates gene expression and cellular function directly, by interacting with DNA via its DNA-binding domain (DBD), and indirectly via interactions with other signaling molecules. To delineate which pathways the AhR invokes to modulate Tfh cells, we used mice with a mutated DBD and demonstrate that the AhR requires its cognate DBD to modulate Tfh cells during IAV infection. All together, these findings provide new information about how the AhR transduces environmental signals to influence the generation of humoral immunity. Given that exposure to AhR-binding pollutants correlates with greater severity of infections and reduced antibody responses to common vaccines, better understanding of the mechanisms that control Tfh cell differentiation and function has broad reaching impact on immune defenses and immune-mediated diseases.

PL 3239 Treatment with AhR Ligands Modulates Concanavalin-Induced T Cell-Mediated Liver Injury by miRNA Dysregulation

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It has been established that immune cells play an important role in the development of many liver diseases, which is a major health problem. A mouse model of immune cell-mediated liver injury that can be compared with Autoimmune Hepatitis in humans by administration of Concanavalin A (ConA) was used to study the effects of aryl hydrocarbon receptor (AhR) ligands. The environmental toxin, 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), an AhR ligand has been shown to promote the differentiation of FoxP3+ regulatory T cells (Tregs), whereas yet another endogenous AhR ligand, 6-formylindolo[3,2-b] carbazole (FICZ) has been shown to exert contrasting effects and promotes proinflammatory Th17 cells. In this study, we tested the hypothesis that AhR ligands are capable of modulating Autoimmune Hepatitis severity by differentially regulating miRNAs to promote or limit these T cell subsets. Mice were injected intravenously with 12.5 mg/kg ConA and then treated with vehicle, 10 µg/kg TCDD, or 50 µg/kg FICZ intraperitoneally, one hour after ConA challenge. Histopathological analysis revealed liver damage in vehicle- and FICZ-treated ConA administered mice but not in TCDD-treated group. Additionally, TCDD-treated mice showed a trend of having lower levels of liver injury enzymes, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) as compared to vehicle-treated ConA exposed mice. Furthermore, flow cytometric analysis of liver-infiltrating mononuclear cells demonstrated that following treatment with TCDD, there was an increase in the percentage and absolute number of FoxP3+CD4+ Tregs as well as a decrease in proinflammatory T bet+ Th1 and RORγt+ Th17 T helper cell subtypes after induction of the disease. In contrast, FICZ did not promote Treg induction. In order to assess the epigenetic mechanisms underlying this T cell dysregulation, total RNA was isolated from liver mononuclear cells and a microRNA microarray was performed. Further, the miRNA expression profile revealed differentially regulated miRNAs such as miR374 associated with T helper transcription factors and cytokines (IL-17). In summary, our data suggests that AhR ligands are capable of dysregulating miRNAs in autoimmune hepatitis, which in turn modulate T cell differentiation to affect the progression of this disease. *Supported by NIH grants P01AT003961, P20GM103641, R01ES030144, R01AI129788 and R01AI123947.*

S 3240 Application of RISK21 Framework in Regulatory-Based Decision-Making: From Business Decisions to Prioritization to Risk Assessment

M. Embry. *HESI, Washington, DC.*

A risk-based approach should be the basic operating principle for decision-making for chemical prioritization and evaluation. An exposure-driven assessment for chemicals proposes a paradigm shift in support of a harmonized risk assessment-based regulatory decision-making. The application of best available science, via integration of new and traditional data streams, will create a risk-based prioritization scheme and tailored exposure-driven risk assessments. A common framework of an integrated approach that enhances efficiency and informs business and risk management decisions that is scientific, transparent, and efficient is available and easy to incorporate into any chemical decision process. The Health and Environmental Sciences Institute (HESI) Risk Assessment in the 21st Century (RISK21; www.RISK21.org) project was initiated to develop a scientific, transparent, and efficient approach to the evolving world of risk assessment. The RISK21 team developed a framework that reconsiders the way chemical risk assessment information is obtained and used. It is a problem formulation-based, exposure driven, tiered data acquisition approach that allows an informed decision on human and environmental safety to be made when sufficient evidence is available and maximizes the ability to inform decisions and optimize resource usage. To achieve innovative solutions in risk evaluation one needs to apply a systematic approach to formulate consistent, technically robust and scientifically credible problems that need to be solved. A structured problem formulation approach enables one to keep focused on describing and evaluating the breadth and depth of the specific problem to be solved, instead of rapidly trying to create solutions. The application of the framework enables the evaluator to identify key issues and possible approaches to address the value of available information and make decisions about what, if any, additional information is needed to inform a decision. This problem formulation-based, exposure-driven, tiered data acquisition approach allows a decision on the sufficiency of available evidence of safety, business risk, or prioritization across a large group of chemicals to be made. The framework enables the assessment of the value of available information and deciding what, if any, additional evidence is needed to reach a decision.

S 3241 High-Throughput Risk-Based Prioritization for Ecological Risk Assessment

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A common approach used in regulatory programs to evaluate chemicals for their potential effects on the environment is to categorize chemicals using various criteria for Persistence, Bioaccumulation, and Toxicity (PBT). Alternative methods screen and prioritize chemicals based on estimates of exposure and potential risk. Recent amendments to the Toxic Substances Control Act (TSCA) have led the US Environmental Protection Agency (US EPA) to consider new approach methodologies (NAMs) in many aspects of TSCA implementation, including prioritization. Much focus has been directed toward *in vitro* toxicity testing. As highlighted in the National Academy of Sciences, Engineering, and Medicine report "Using 21st Century Science to Improve Risk-Related Evaluations," there is a need to develop and evaluate exposure models to compare against existing and emerging data streams of toxicity information to guide risk-based decision-making. The Risk Assessment IDentification And Ranking (RAIDAR) model combines chemical fate and transport at a regional scale with bioaccumulation in aquatic, terrestrial, and agricultural food webs using toxicokinetic models to estimate exposures to a wide range of representative ecological receptors. User-defined toxicity data can then be compared with exposure estimates to quantify risk. To demonstrate high-throughput risk-based prioritization for ecological health, the RAIDAR model was applied as a case example to 11,000 organic chemicals and the results were evaluated using the RISK21 matrix tool. The risk-based estimates span more than 10 orders of magnitude, demonstrating the capacity to screen and prioritize large numbers of chemicals for ecological risk. Uncertainty analysis further demonstrates the greatest overall source of uncertainty in the prioritization is the emission rate estimate used as input into the model. The results of the RAIDAR calculated risk estimates and ranking for chemical prioritization are compared against traditional "PBT" categorization results, highlighting the need for considering NAMs in regulatory decision-making.

S 3242 Exposure- and Hazard-Driven Prioritization for Evaluation of Chemicals in Canadian Drinking Water

I. Moffat. *Health Canada, Ottawa, ON, Canada.* Sponsor: M. Embry

Health Canada, in collaboration with the Federal-Provincial-Territorial Committee on Drinking Water, develops and publishes the *Guidelines for Canadian Drinking Water Quality* for chemical and microbiological parameters. For chemicals, the guidelines specify the maximum acceptable concentrations of a chemical(s) in drinking water. As part of our cyclical five-year prioritization process, a list of 421 chemicals was identified for potential evaluation (based on new science, existing priorities, emerging concerns, monitoring, sales/use, and reviews by other agencies). Our goal was to identify which of these 421 chemicals have the highest priority for evaluation. Health Canada used the RISK21 framework (HESI, US), including problem formulation, which integrates tiered exposure and hazard information into a risk plots for iterative rounds of decision-making, ultimately using the appropriate tier to decide on a chemical's priority. Exposure information relied on national and global exposure databases. Tier 1 exposure information included physi-

cochemical properties, and Tier 2/3 information was from biomonitoring and monitoring databases (examples will be provided in the presentation). For hazard characterization, Tier 1 information was from published reference values, while the later tiers used predictor information (e.g., extrapolation/activity models, apical endpoints, and mode-of-action analysis). Iterative rounds of exposure and hazard information from appropriate tiers were used to generate risk plots (exposure versus toxicity) until a decision (high, low, wait) could be made on all chemicals. We prioritized 62 high, 290 low, and 69 wait priority chemicals for further evaluation. Using this tiered system, we efficiently used resources and precision to make a decision to rank the priority of all chemicals. This prioritization process provides a science-based, methodologically robust, and transparent way to identify and prioritize chemicals/groups requiring the development of a drinking water risk assessment.

3243 Using the RISK21 Framework as a Tiered Approach for Chemical Risk Assessment: A Proof of Concept

S. Deglin. *HESI, Washington, DC.* Sponsor: M. Embry

This presentation will ground-truth the RISK21 approach through the systematic and tiered risk assessment of a large number of chemicals. The purpose of this example is to illustrate the advantages of a tiered approach by comparing risk-based estimates of lower-tier, less resource-intensive methods with the higher-tier, more traditional information sources. This work builds upon initial evaluations performed by Wetmore et al. (2015) and Patlewicz et al. (2018), where numerous chemicals were evaluated using high-throughput exposure (HTE) predictions combined with either oral equivalent doses (OEDs) derived from dosimetry-adjusted *in vitro* bioactivity data from ToxCast (Wetmore) or the Threshold of Toxicological Concern (TTC) approach (Patlewicz). In this case study, the RISK21 approach is applied to over 7,000 chemicals from the previous studies to verify that the framework is valid, and protective, of human health. First, the TTC approach and HTE values were applied as a Tier 0, and most conservative assessment. The Cramer class of each chemical was determined using Toxtree, and the corresponding toxicity values and HTE estimates from previous studies were plotted on the RISK21 matrix. OED values from ToxCast data were then combined with the HTE estimates to evaluate the relative conservativism between the two approaches (TTC + HTE versus OED + HTE). As a third step, *in vivo* hazard data from traditional animal studies were used in concert with the HTE estimates to further evaluate the tiered approach. The most conservative No-Observed-Adverse-Effect Level (NOAEL) values were used in this last phase. This case study showed that the RISK21 framework is a valid approach to evaluate risk at various levels of refinement. Although both the TTC and ToxCast approaches present some limitations that need to be accounted for upfront when dealing with certain chemistries such as bioaccumulative chemicals, metals, and inorganic substances for TTC, and volatiles, some surfactants, and non-DMSO soluble substances for ToxCast.

3244 Use of RISK21 for Communication of Absolute and Comparative Risks: The Case of Glyphosate Use in Different Countries

A. Moretto. *Università degli Studi di Milano, Milan, Italy.*

The RISK21 framework was used to evaluate glyphosate risk to the general population in Italy (and the broader European Union), Argentina, and Brazil. Exposure estimates were based on available water and food monitoring data, and an estimate of farmworker exposure was performed using European exposure models. Monitoring data have been collected for water and food over the years, and glyphosate intakes have been estimated both deterministically and probabilistically, based on national food consumption data and the default assumption on drinking water intakes. Acceptable daily intakes (ADI) as set by the Joint FAO/WHO Meeting on Pesticide Residues, the European Food Safety Authority (EFSA), and the US Environmental Protection Agency using the standard safety factor of 100 (i.e., a margin of exposure [MoE] from the critical NOAEL) have been used. For the European scenario, the Acceptable Operator Exposure Level was compared with the estimated exposure based on the EFSA models. Exposures of both the general population and the farmer applying glyphosate were well below the health-based limits. This information was graphically visualized in the RISK21 matrix and contrasted with similar assessments performed for other herbicides, possible substitutes of glyphosate, which proved to pose a higher risk. The use of the RISK21 matrix helps to clearly present the conclusion to the stakeholders, including the less technically prepared general population.

3245 Application of the RISK21 Framework as a Decision and Communication Support Tool to Inform Project Risk and Business Decisions in Crop Protection Active Substance Development

D. Cowie. *Syngenta, Bracknell, United Kingdom.* Sponsor: M. Embry

A modern approach to informing agrochemical product development decision-making applies the RISK21 framework to incorporate new approach methods and predictive tools to determine data needs to deliver registerable products and inform re-registration decisions. Using problem formulation directs the process for determining what exposure and hazard data are necessary to develop in order to adequately inform each step of the new product development or respond to issues that arise during re-registration discussions. The presentation will provide examples of problem formulation focused on risk management decisions that drive studies to assess relevant exposure and hazard concerns to enable risk-based decisions for safe use.

3246 Chemical Probes in Toxicology: From Defining Exposures to Identifying Novel Toxicity and Druggable Targets

J. Smith. *Pacific Northwest National Laboratory, Richland, WA.*

As an emerging scientific discipline, chemical biology utilizes synthetic chemical probes to functionally identify and measure reactive biological molecules such as proteins and nucleic acids. Researchers design and synthesize small molecule chemical probes to functionally target and covalently label enzymes, receptors, and nucleic acids based on catalytic activities and selective affinities. Combined with fluorescence gel electrophoresis, liquid chromatography-mass spectrometry-based proteomics, and bioinformatics, chemical probe platforms facilitate rapid and quantitative screening of cells, tissues, and biological fluids from microbes, animal models, and humans. Compared with conventional transcriptomics and proteomics, chemical probes provide measurements of functional activity rather than total abundance of transcripts, proteins, or nucleic acids. Thus, chemical probes have recently gained popularity among research toxicologists and drug developers as tools to measure enzymatic activity important in metabolism and identify novel molecular binding targets of toxicants and drugs. The session will highlight innovative methods using chemical probes in the field of toxicology. The first speaker will present how chemical probes can measure and predict metabolism and internal dosimetry. This will be followed by a demonstration on how chemical probes can be used to identify novel targets of organophosphates beyond acetylcholinesterase inhibition. The next presentations will discuss how chemical probes can reveal chemically induced damage to DNA and resulting mutations and how chemical probes can be used to reveal novel druggable targets and possible toxicity targets. The final speaker will demonstrate how chemical probe techniques can identify off-target proteins and mechanisms of a fatal experimental drug.

3247 Applying Activity-Based Protein Profiling to Better Measure and Predict Metabolism and Internal Dosimetry

J. Smith. *Pacific Northwest National Laboratory, Richland, WA.*

As one of the primary processes controlling internal dosimetry, metabolism plays a key role in (1) bioactivating xenobiotics to form an ultimate toxicant or (2) clearing and detoxifying toxicants post-exposure. Conventional methods measuring xenobiotic metabolism rely on descriptive experiments measuring parent disappearance or product formation in cell culture systems, subcellular fractions, or isolated enzyme systems. *In vitro* to *in vivo* methods translate metabolism measurements to applicable test systems (e.g., cell culture, zebrafish, animal model, humans) for internal dosimetry congruence, improving hazard identification and dose-response analysis. This talk will present novel approaches using activity-based protein profiling to better measure and predict metabolism and internal dosimetry across test systems using polycyclic aromatic hydrocarbons (PAHs). As classical procarcinogens, many PAHs require metabolic bioactivation to form the ultimate toxicant and metabolic detoxification for compound clearance. Our group has developed and applied activity-based probes of cytochrome P450s and glutathione S-transferases to identify enzymes involved with metabolism of PAHs, quantify enzyme induction from PAH exposures, measure implications of enzyme induction on PAH metabolism, and measure enzyme ontology in humans. Combining activity-based protein profiling methods with classical metabolism assays and physiologically based pharmacokinetic (PBPK) models allow for novel

approaches for *in vitro* to *in vivo* extrapolation of metabolism across systems and predictions of metabolism rates across human life stages and population variability. *Funded by NIEHS Grant No. P42 ES016465.*

3248 Structure-Dependent Determination of Organophosphate Targets in Mammalian Tissues Using Activity-Based Protein Profiling

V. Lin. *Pacific Northwest National Laboratory, Richland, WA.* Sponsor: J. Smith

Acute and chronic exposure to organophosphates (OPs), including agricultural pesticides, industrial chemicals, and chemical warfare agents, has significant short-term and long-term impacts on human health. The mechanisms by which OPs alter development and cognition in exposed individuals remain poorly understand, in part due to the large number of structurally diverse OPs that are used worldwide and the wide range of affected proteins and signaling pathways. To investigate the influence of structure on OP targets in mammalian systems, we developed a series of OP probes for activity-based protein profiling (ABPP) featuring two different reactive groups for mimicking OP chemical reactivity. ABPP using these new probes in mouse liver and brain revealed divergent protein profiles, demonstrating that probe structure influences identified protein targets. Competition experiments using paraoxon and chlorpyrifos oxon also showed that the probes can be used to detect differences in sensitivity toward OPs among protein targets in a complex mixture. Additionally, these probes can be used to assess reactivation of acetylcholinesterase by pralidoxime (2-PAM) and may serve as diagnostic tools for screening of therapeutic candidates in a panel of protein targets. These applications will help clarify the effects of OP toxicity beyond acetylcholinesterase inhibition, investigate potential points of convergence for broad spectrum therapeutic development, and support future efforts to screen candidate molecules for efficacy in various model systems.

3249 Chemical Probes to Uncover Genome-Wide Patterns of Chemically Induced DNA Damage and Mutagenesis

S. Sturla. *ETH, Zurich, Switzerland.*

Exposures to an increasing variety and amount of chemicals from the environment, diet, and drugs, together with defects or deficiencies in DNA damage tolerance pathways, contribute to carcinogenesis risk. Current understanding of how DNA oxidation and alkylation drive mutagenesis is advanced, but our ability to predict the mutagenicity of chemicals or disease risks related to these processes remains limited, in part because there exists a mismatch between our low-resolution understanding of how DNA adducts are distributed and dynamically altered on a genome-wide level versus our sophisticated knowledge of mutational landscapes of human cancers. Systems toxicology models linking chemical exposures with mutational signatures are fundamentally limited by this lack of information and methods to map DNA damage. The objectives of this work are to map oxidation and alkylation damage in mammalian cells, understand how damage maps are governed by chemical sources and repair, and relate chemical exposures with *in vitro* mutation signatures. To address these goals, we have developed chemical probe-based strategies for mapping damage regions in the human genome. Results include development and application of a method for single nucleotide mapping of damage in whole genomes and *in vitro* strategies to determine the impact of enzymatic deficiencies on mutation signatures. We found that the specificity of DNA repair enzymes could be coupled with efficient click DNA ligation reactions to insert a biocompatible nucleoside probe with a locator oligonucleotide code sequence. This approach enabled high-throughput, nucleotide-resolution sequencing of DNA damage. We mapped thousands of damage sites with distinct patterns related to transcription, chromatin architecture, and chemical oxidation potential. Furthermore, we found that loss of the capacity to extend DNA synthesis from methylnitrosourea-induced damage sites produced altered DNA mutation signatures. These sequence-specific chemical probe-based strategies for tracking chemically induced DNA damage overcome previous limitations to sequencing of DNA damage, provide information concerning the relationship between DNA repair enzyme function and mutation signatures, and could lead to new approaches for quantifying risk on the basis of early biomarkers and characterization of causative factors of individual cancers.

3250 Utilizing Chemoproteomic Platforms to Elucidate Toxicological Mechanisms

B. Ford. *US EPA, Research Triangle Park, NC.* Sponsor: J. Smith

A large number of pharmaceuticals, endogenous metabolites, and environmental chemicals act through covalent mechanisms with protein targets, yet the specific interactions with the proteome remain poorly defined for many of these reactive chemicals. Deciphering direct protein targets of reactive small-molecules is critical in understanding their biological action, off-target effects, and potential toxicological liabilities, as well as for the development of safer and more selective chemical agents. Chemoproteomic technologies have arisen as a powerful strategy that enables the assessment of proteome-wide interactions of these irreversible agents directly in complex biological systems. This talk will focus on the utilization of the powerful chemoproteomic approach termed Isotopic tandem orthogonal proteolysis-enabled activity-based protein profiling (isoTOP-ABPP) in identifying the direct protein binding, possible targets, mechanism of toxicity and target selectivity of environmental chemicals. The research discussed was all performed as within an academic setting and will demonstrate the potential of chemoproteomic platforms for understanding the mechanism of toxicity, off-targets, selectivity and potential of the platform as part of the covalent compound discovery and development process towards assessing and developing safer chemicals.

3251 Activity-Based Protein Profiling Reveals Off-Target Proteins of Fatal Experimental Drug BIA 10-2474

M. van der Stelt. *Universiteit Leiden, Leiden, Netherlands.* Sponsor: J. Smith

A recent phase 1 clinical trial of the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474 led to the death of one volunteer and produced mild-to-severe neurological symptoms in four others. Although the cause of the clinical neurotoxicity is unknown, it has been postulated, given the clinical safety profile of other tested FAAH inhibitors, that off-target activities of BIA 10-2474 may have played a role. Here, we use activity-based proteomic methods[1] to determine the protein interaction landscape of BIA 10-2474 in human cells and tissues. This analysis revealed that the drug inhibits several lipases that are not targeted by PF04457845, a highly selective and clinically tested FAAH inhibitor. BIA 10-2474, but not PF04457845, produced substantial alterations in lipid networks in human cortical neurons, suggesting that promiscuous lipase inhibitors have the potential to cause metabolic dysregulation in the nervous system.[2] *References: 1. Van Rooden et al.,* Nature Prot*. 2018, 13, 752. 2. Van Esbroeck et al.,* Science*, 2017, 356, 1084.*

3252 Express Yourself (or Not . . .): the Nonclinical Safety of Oligonucleotide Therapeutics

J. Sutherland. *Alnylam Pharmaceuticals, Cambridge, MA.*

Oligonucleotide-based therapeutics that utilize nucleic acids to treat a variety of local and systemic diseases are very novel and relatively unknown to the public. This unique class of agents includes antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), aptamers, microRNA (miRNA) inhibitors and mimics, and modified messenger RNA (mRNA), which usually require special systems for delivery to patients and specific safety considerations. In the last 20 years, several ASOs have been approved for the treatment of serious diseases, including fomivirsen for cytomegalovirus retinitis; mipomersen for homozygous familial hypercholesterolemia; nusinersen for spinal muscular atrophy; and inotersen for polyneuropathy caused by hereditary transthyretin-mediated (hATTR) amyloidosis. In 2018, patisiran, the first siRNA therapeutic, was approved for the treatment of polyneuropathy of hATTR amyloidosis. A robust nonclinical and clinical pipeline features other ASOs, siRNAs, and modified mRNAs designed to treat a variety of systemic and local diseases. The goals of this Symposium are to provide an update on the current status of the ASO, siRNA, and modified mRNA platforms; their respective mechanisms of action; their chemistry; and some of the challenges each modality must overcome to enable effective systemic and localized delivery to target tissues, with an acceptable benefit-to-risk profile. Pharmaceutical development of these agents constitutes a prime example of bridging cutting-edge research with the established regulatory requirements allowing for clinical testing. Effective nonclinical safety assessment and the regulatory requirements for evaluating the risks and benefits of these molecules, including appropriate species selection and determination of safety margins, are of paramount importance in bringing them to the clinic as novel therapy options to patients. After this session, attendees will have a better understanding of the nonclinical safety characteristics and development strategies of this important emerging class of oligonucleotide-based therapeutics.

3253 What Do I Do with All This Data? Mining the Database of 2′-MOE ASO Monkey Toxicology Studies

S. P. Henry. *Ionis Pharmaceuticals, Carlsbad, CA.*

The development of ASOs has been a journey of optimizing chemistry, sequence, and screening methods to obtain the best drug candidates. The 30 years of ASO development experience have produced an unprecedented amount of data on a specific drug platform documenting the behavior in animals that broadly informs safety monitoring in clinical trials. Individual animal and time point data from toxicology studies have been collected into databases that represent >2,000 monkeys treated with 2′-MOE ASOs and >600 monkeys treated with GalNAc-conjugated 2′-MOE ASOS. This collective experience is particularly useful as the diversity of antisense applications expands with different routes of administration, chemical modifications, and tissue-targeting strategies. This database has proven to be useful in a number of ways, including better characterizing the dose-response for severe thrombocytopenia. The large datasets also have facilitated the overall safety evaluation of hepatic tolerability of GalNAc-conjugated 2′-MOE ASOs, compared with their unconjugated parent molecules. The ability to draw from data that represent multiple closely related compounds facilitates the interpretation of the relevance of findings to humans and safety margins. This is particularly important in the case of low incidence findings in toxicology studies that are limited to a relatively small number of animals. Thus, these databases help inform the appropriate safety monitoring in clinical trials within the context of the disease background. There also is an opportunity to use these data to tailor the nonclinical safety program to minimize animal use while efficiently supporting the investigation of rare diseases.

3254 Nonclinical Safety Assessment of siRNA Therapeutics

J. A. Dybowski. *Alnylam Pharmaceuticals Inc., Cambridge, MA.*

Short interfering RNA (siRNA) molecules are a new class of human therapeutics that selectively target the endogenous RNA interference mechanism controlling the translation of RNA to protein. Through this mechanism, siRNA therapeutics can be designed to specifically target mRNA and silence the production of disease-associated proteins. siRNA therapeutics are synthetic double-stranded RNA molecules that have been chemically modified (e.g., alterations to sugar moiety, phosphate backbone, nucleobase, termini, and conjugation groups) to enhance stability and specificity. Intracellular drug delivery of these highly polar compounds requires the use of encapsulating formulations (e.g., lipid nanoparticles) or the conjugation to ligands (e.g., N-acetylgalactosamine [GalNAc]) for receptor-mediated endocytosis. There is a lack of specific regulatory guidance for the safety assessment of oligonucleotides. The unique physical, chemical, and pharmacological properties of siRNA present challenges to the design of a nonclinical safety testing strategy that requires the leveraging of guidance for both large and small molecule therapeutics. This presentation will provide an overview with examples of the factors that need to be taken into consideration when designing a nonclinical toxicology testing strategy, including species selection, appropriate use of surrogate compounds, and impact of ADME properties. Additionally, an overview summarizing the toxicological properties of siRNA therapeutics commonly observed in nonclinical species will be provided along with a discussion of the relevance of these findings to patient safety.

3255 Delivering on the Promise of mRNA Therapeutics Safely and without Complement

J. J. Senn. *Moderna Inc., Cambridge, MA.* Sponsor: J. Sutherland

mRNA-based therapies have enormous potential. Nonetheless, how to combat mRNA's inherent biological lability, how to direct therapeutic mRNAs to desired cell types, and how to enable repeat dosing without eliciting adverse immune reactions remain significant challenges for the field. Lipid nanoparticles (LNPs), consisting of an ionizable lipid, a phospholipid, cholesterol, and a PEGylated lipid, are our current method of choice for packaging and *in vivo* delivery of therapeutic mRNAs. First-generation mRNA-LNPs enabled both proof of concept preclinical studies and early clinical trials demonstrating mRNA-based vaccine efficacy. Other uses of mRNA-LNPs have been hampered, however, by dose-limited toxicities and an inability to repeat dose upon IV administration. Like other PEGylated particles, PEGylated LNPs are known to induce complement activation upon bolus dosing, which can result in systemic inflammation and liver injury in rodents and systemic inflammation and anaphylactoid responses in swine and nonhuman primates. Although these effects can be ameliorated by long duration (> 60 min) infusions, an alternate means of administration is highly desirable. PEGylated mRNA-LNPs also are recognized by the immune system via IgM and IgG-mediated responses that both activate complement (causing toxicity) and redistribute them to the Reticuloendothelial System (RES), diminishing protein production by accelerated blood clearance. Over the past several years, we have invested significant effort into developing next-generation mRNA-LNPs with improved characteristics, such as better biodistribution to desired tissues and improved tolerability. Here, we describe new PEGylated lipids that substantially reduce both complement activation and accelerated blood clearance. Our propriety mRNA-LNPs containing these new PEGylated lipids allow for repeat IV bolus dosing with significantly increased therapeutic index compared with industry standards.

3256 Oligonucleotide Therapeutics: US FDA Experience and Regulatory Consideration

X. Chi. *US FDA/CDER, Silver Spring, MD.* Sponsor: J. Sutherland

This presentation will provide an overview of the oligonucleotide (ONT) regulatory landscape in terms of current guidance applicability and some special considerations based on US Food and Drug Administration (US FDA) experience with various classes of ONT submissions. ONT submissions to the Center for Drug Evaluation and Research (CDER) at the US FDA have increased substantially over the years. Over 300 ONT Investigative New Drug (IND) submissions and 16 New Drug Applications (NDAs) have been received by the center in the past 27 years, and the submissions span a wide variety of ONT classes, routes of administration (ROAs), and therapeutic areas. The changing trend in ONT classes and ROAs reflect the need for targeted organ delivery and strategies to reduce systemic toxicities. ONTs lie at the interface between small molecules and biologics in terms of physiochemical and toxicological properties. The nonclinical support required for clinical development tends to be determined case by case, and a hybrid of ICH M3(R2) and ICH S6 is generally applied. The requirement also is adapting to evolving knowledge and accumulating data in the field. For general toxicology evaluation, the two species rule applies and at least one species should be pharmacologically relevant. The CDER Pharmacology/Toxicology Coordinating Committee—ONT Subcommittee is developing an ONT Nonclinical Database on data submitted to the center, with a focus on dose-limiting toxicities stratified by ONT classes and translatability of nonclinical findings. Once clear patterns are identified, they can be used to help further inform regulatory decisions or contribute to the development of future position papers or guidance documents.

3257 Identifying and Modeling Gene-Environment Interactions in Neurological Diseases Associated with Metal Exposure: Challenges and Recent Advances

O. Adebambo. *Duke University, Durham, NC.*

Epidemiological studies have revealed that the interplay between genetic background and the environment can contribute to disease onset and severity. Even disorders that are recognized as straightforward Mendelian diseases have been shown to display substantial phenotypic variability attributable to multifactorial interactions, such as those between genes or the gene and the environment. With more etiologically heterogeneous disorders such as neurological diseases, these interactions are even more complex and accurately investigating them in a laboratory setting has proven challenging. To address these problems, the goal of this session is to examine experimental methodologies that are currently being used in the field to model and investigate the link between genetics and environmental exposures in neurologic diseases. In this context, environmental metal exposure is of particular interest because excessive metal levels can accumulate in the brain, thereby leading to detrimental intracellular events that can alter neurotransmission and lead to neurodegeneration. Thus, we will bring together experts to discuss the influence of exposure to metals and particulate matter on known nonpenetrant genetic mutations in Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Parkinson's disease (PD) using *in vitro*, mammalian, and nonmammalian experimental models. Additionally, the panel of presenters were selected based on their utilization of distinct modeling strategies so as to enrich the discussion with a diversity of perspectives. We will answer questions such as: How do external environmental factors gain access to the nervous system, alter its elements, and interact with the genome to generate disease? What experimental mammalian and nonmammalian animal models are available for interaction modeling? How can we use novel genetic engineering techniques such as CRISPR/Cas9 to generate humanized animal models to better investigate genetic-environment interactions that can be extrapolated to humans? What constitutes an actual gene exposure interaction versus additivity of stressors, and can we distinguish between them? Are there phenotypic or disease severity differences based

on timing of exposure? Does aging modulate the effect of metal exposure on neurodegenerative disease onset? After the session, attendees will have a better understanding of experimental models and tools available for modeling gene-environment interactions in neurologic diseases and how to circumvent the underlying challenges in implementing these studies. Furthermore, they will gain perspective on factors for consideration during experimental design, such as timing of exposure and determining susceptibility windows and aspects of data rigor and reproducibility.

S 3258 Age- and Manganese-Dependent Modulation of Dopaminergic Phenotypes in a *C. elegans* DJ-1 Genetic Model of Parkinson's Disease

M. Aschner. *Albert Einstein College of Medicine, Bronx, NY.*

Parkinson's disease (PD) is the second most common neurodegenerative disease, yet its etiology and pathogenesis are poorly understood. PD is characterized by selective dopaminergic (DAergic) degeneration and progressive hypokinetic motor impairment. Mutations in protein deglycase (DJ-1) cause autosomal recessive early-onset PD. DJ-1 is thought to protect DAergic neurons via an antioxidant mechanism, but the precise basis of this protection has not yet been resolved. Aging and manganese (Mn) exposure are significant nongenetic risk factors for PD. Modeling aging (A) and its interaction with genetic susceptibility (G) and environmental exposure (E) can be challenging in animals, but *Caenorhabditis elegans* (*C. elegans*) is an optimal model for PD (AXGXE) studies because of its simple nervous system, conserved DAergic machinery, and short 20-day life span. Here, we tested the hypothesis that *C. elegans* DJ-1 homologues were protective against Mn-induced DAergic toxicity in an age-dependent manner. We showed that the deletion of *C. elegans* DJ-1 related (djr) genes—djr-1.2 that is expressed in DAergic neurons—decreased survival, life span, and dopamine (DA)-dependent dauer movement after Mn exposure. We also tested the role of DAF-16 as a regulator of dj-1.2 interaction with Mn toxicity. Lifespan defects resulting from djr-1.2 deletion could be restored to normal by overexpression of either DJR-1.2 or DAF-16. Furthermore, dauer movement alterations after djr-1.2 deletion were abolished by constitutive activation of DAF-16 through mutation of its inhibitor, DAF-2 insulin receptor. Taken together, our results reveal PD-relevant interactions between aging, the PD environmental risk factor manganese, and homologues of the established PD genetic risk factor DJ-1. Additionally, our data demonstrate a novel role for the DJ-1 homologue, djr-1.2, in mitigating Mn-dependent life span reduction and DA signaling alterations, involving DAF-2/DAF-16 signaling. In this context, the experimental modeling strategies to account for interspecies differences and extrapolation to human PD pathogenesis will be discussed. Furthermore, the incorporation of rigor to ensure robust and unbiased experimental design that has resulted in reproducibility of our findings in *C. elegans* and other mammalian systems also will be examined.

S 3259 Utilizing a Knock-In Mouse Model of Disease to Evaluate the Effect of Gene-Environment Interactions on Cognitive Impairment Associated with Alzheimer's Disease

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Environmental factors and gene-environment interactions (GXE) may increase Alzheimer's disease (AD) risk and accelerate cognitive decline. However, there is a paucity of information directly supporting this hypothesis. The goal of our study is to test this hypothesis in an animal model. Knock-in mouse models are particularly advantageous in this context because they enable us to create humanized mouse models in which the endogenous mouse gene is replaced by the human gene. Apoliprotein E4 (ApoE4) is the strongest genetic risk factor for late-onset AD, while ApoE3 is the most common allele of apolipoprotein in humans. The presence of the ApoE2 allele reduces AD risks. We assessed for a GXE between ApoE and environmental exposure to heavy metals (lead, cadmium) or diesel exhaust on cognitive behavior using transgenic knock-in (KI) mice that express the human ApoE4, ApoE3, or ApoE2 under the control of the endogenous mouse ApoE promoter. Lead and cadmium are heavy metals and ubiquitous environmental contaminants that pose significant public health concern globally. Young adult animals were exposed to lead or cadmium that yielded blood concentrations that are relevant to current human exposure. Separate cohorts of animals also were exposed to diesel exhaust-generated $PM_{2.5}$ at levels relevant to human exposure. Sample sizes of n=10-12 animals were used for each genotype and treatment group. We performed behavior tests to monitor learning and memory, and to elucidate underlying mechanisms, we also examined the effect on hippocampal adult neurogenesis. Statistical analysis was performed to examine the presence of GxE interaction. Our data suggest that GXE between ApoE4 and environmental exposure contribute to cognitive impairment, while ApoE2 may be protective. Furthermore, impaired adult hippocampal neurogenesis may contribute to the observed deficits in cognitive behavior.

S 3260 Manganese-Induced AKT Signaling Occurs via Direct Effects on Insulin Growth Factor Receptor Signaling and is Impaired in Huntington's Disease

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Manganese (Mn) is an essential metal that is neurotoxic in excess. In this study, we assessed the effects of Mn exposure, and the altered Mn handling in Huntington's disease (HD) models, on insulin/insulin-like growth factor (IGF) signaling. Mn has been linked to insulin homeostasis and signaling, but the mechanism is unresolved. In this talk, we report that even at saturating concentrations of IGF1 (10 nM), high intracellular Mn levels (50 μM-500 μM exposures for 24 hrs) potentiates IGF-induced p-AKT in STHdh, PC12, and hiPSC-derived neuroprogenitor cell (NPCs) culture models, suggesting that elevated Mn levels can increase maximal insulin/IGF receptor activity and phosphorylation. Furthermore, chronic seven-day exposures of cells at environmentally relevant levels (1 μM-10 μM Mn) also activated AKT signaling, which is downstream of insulin/IGF receptors. Both the chronic and 24-hour Mn-dependent signaling effects are suppressed in the HD genotype, consistent with reports of an HD by Mn interaction effect. Given the synergy between Mn and insulin/IGF-receptors (IR/IGFR), we further hypothesized that Mn-induced p-AKT may be directly IR/IGFR dependent. Consistent with this hypothesis, >70% of Mn-induced p-AKT can be blocked with IR/IGFR inhibitors (BMS-536924, BMS-754807, Linsitinib [OSI-906], NVP-AEW541), demonstrating that Mn-induced p-AKT is almost entirely dependent on these receptors and that intracellular Mn, not extracellular Mn, is exerting these effects. To assess if Mn-induced IGF signaling facilitates relevant functional responses, we examined glucose uptake and found HD reductions in glucose uptake were selectively increased by Mn exposure. Collectively, our data suggest that (1) the majority of the known Mn-induced p-AKT response is IR/IGFR dependent versus other signaling pathways upstream of AKT activation, (2) Mn acts intracellularly to modify function of the insulin/IGF receptors, and (3) HD-associated changes in Mn homeostasis may underlie known HD reductions in IR/IGFR signaling. This work provides evidence that deviations in cellular Mn homeostasis by exposure or HD genotype effects may exert their pathophysiological effects via altered function of Mn-dependent cell signaling.

S 3261 Modeling Genuine Gene-Environment Interaction and Not Additivity in Amyotrophic Lateral Sclerosis (ALS): The Dilemma between "Real-World" Exposure/Genetic Makeup and Mouse-to-Human Translation

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ALS is the most common adult-onset paralytic disorder, usually fatal within one to five years after symptom onset. Most previous ALS GXE studies have used animal models with a full-blown phenotype and environmental exposures already significantly neurotoxic by themselves, questioning whether GXE or G+E were actually studied. ALS patients do not usually report any signs of neurotoxicity before developing the paralytic disease, suggesting that genuine GXE interaction in ALS most likely results from the interaction between silent exposures and silent genetic susceptibilities. Therefore, a knock-in mouse model (on a C57BL/6J background) of an incompletely penetrant variant of TDP-43 "G298S" was used to model this hypothesis. The heterozygous (het-G298S) knock-in mice are "ALS-silent" over their life span, but the homozygous knock-in show mild neuromuscular junction denervation at an advanced age (2.5 years), confirming the validity of these models. Primary spinal cord cultures from het-G298S mice were generated to screen for possible GXE interaction with various neurotoxic metals previously proposed to have a role in ALS. This pilot *in vitro* study identified that G298S increased the vulnerability of motor neurons to manganese (Mn) exposure. Subsequently, wild-type (WT) and het-G298S mice were chronically exposed via drinking water to 200 ppm Mn (a level of exposure rarely met in human exposure but comparatively lower than used in previous mouse studies) for six months starting in young adulthood (P90). To control for sex effect, this study included 50% females and 50% males, and it was powered based on our past ALS mouse studies. Mn-exposed and control mice were tested weekly by treatment/genotype-blinded investigators for grip strength (loaded grid) or general locomotor activity (rotarod) over the six months of the study. At the end of the study, mice also were tested with CatWalk for gait and quantitative assessment of footfalls. Some mild motor deficits were observed in het-G298S mice, but no phenoconversion to an ALS phenotype was observed in relation

to Mn treatment. However, these are in contrast to data currently being obtained from a separate cohort of het-G298S and WT mice exposed to higher concentrations (400 ppm) of Mn, for which the installation of a GXE and progressive ALS-relevant motor deficits are observed.

3262 Modeling Gene-Environment Interactions in Manganese Overexposure Using Dopaminergic Cell Culture and MitoPark Model of Parkinson's Disease

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The preferential accumulation of manganese (Mn) in the basal ganglia induces death of pyramidal neurons, with processes paralleling that of dopaminergic cell death in Parkinson's disease (PD). However, it is not clear if Mn contributes to PD etiology. Mounting data have pointed to mitochondrial dysfunction as a major contributor to neurodegeneration. Coincidentally, exposure to heavy metals such as Mn could impair mitochondrial bioenergetics via accumulation in the mitochondria. Thus, genes important for mitochondrial bioenergetics and biogenesis found to be dysregulated in PD postmortem brains and PD animal models, namely mitochondrial transcription factor A (TFAM) and prokineticin 2 (PK2), have since come under scrutiny. To investigate the link between downregulation of these prosurvival genes involved in PD and susceptibility to Mn exposure, PK2 and TFAM knockout (KO) dopaminergic cell lines were generated using CRISPR/Cas9. The goal was to investigate the mechanism by which these prosurvival genes act during Mn neurotoxicity and to examine whether Mn exposure augments mitochondrial dysfunction. Both PK2-KO and TFAM-KO dopaminergic neuronal cells were more vulnerable to Mn toxicity than control cells at 300 μM Mn, as shown by increased cell death after 24 h of treatment, and exhibited statistically significant decrease in mitochondrial basal and ATP-linked respiration. To further validate these results *in vivo*, we utilized a newly available transgenic mouse model, the MitoPark mouse, which exhibits mitochondrially defective dopaminergic neurons via Cre-LoxP conditional KO of TFAM. Male and female MitoPark mice were randomly selected for Mn treatment. We found that exposure to a physiologically relevant dose of Mn (10 mg/kg for 30 days) caused a statistically significant increase in nigral dopaminergic neuronal loss and accelerated disease progression in MitoPark mice, with further exacerbation of oxidative damage in the striatum and substantia nigra. Our results collectively indicate the ability of heavy metals such as Mn to augment PD disease state in a rodent model of PD while demonstrating that CRISPR/Cas9 gene editing can be used as an excellent method to model gene-environment interactions associated with mitochondrial defects in dopaminergic neurons.

3263 Protecting the Force: Examining the Hazards of Military Service from the Bench and the Battlefield

M. Johnson. *Army Public Health Center, Aberdeen Proving Ground, MD.*

It is estimated that more than 20 million US citizens either have served or are currently serving in the military. Therefore, in addition to preparing a force that is ready to meet future challenges and threats to ensure national security, protecting the health of the service members and veterans is and must continue to be a national priority. Military service involves a vast and unique set of toxicological considerations when compared with most occupational settings, which varies for each individual. These include, but are not limited to, exposures related to the use of military-specific materials, hazards that are characteristic of the duties and/or locations of service (e.g., deployments), and the physical consequences of combat. A thorough understanding of the potential impacts of these experiences on health (both short- and long-term) is required. This information also is vital in preparing for future missions and ensuring the safety of service members and quality of life of veterans. As representative examples, the speakers in this session will discuss (1) the systemic and local effects of embedded metal fragments from blast injuries, (2) the identification of subtle respiratory dysfunction related to exposure to airborne hazards during deployment, and (3) evaluations of toxicity and long-term health implications due to jet fuel exposure. These topics will highlight some hazards and exposure pathways specific to military personnel, a large population with a unique and complex occupational environment, carrying out operations to sustain national security, and the challenges involved in maintaining their health during and after their service.

3264 Tungsten Alloys in Tissue, Biocorrosion, and Tumor Outcome

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Military and civilian personnel in areas of conflict can be exposed to metal fragments from bullets or explosions. Depending on the size, body location, and risks associated with surgical removal, these fragments can remain in tissue for years, or even a lifetime. The potential toxicity and health effects of long-term exposure are therefore of concern. Tungsten alloys, which are used in munitions, are composed of tungsten microparticles embedded in a solid matrix of transition metals that can include nickel, cobalt, and iron. Intramuscular implants in rodents with different pellet compositions have been carried out by various groups, resulting in a range of sarcoma incidences. Our work has shown that alloy composition and biocorrosion of component metals are important determinants of health outcomes, while the species of rodent used also is a factor in tumor response to metal alloys. This presentation will summarize the field to date regarding studies on potential toxicity of tungsten alloys and identify future research needs.

3265 Metal Exposure in Veterans with Embedded Fragments from War-Related Injuries: Exposure Assessment and Surveillance Outcomes

J. M. Gaitens, and M. A. McDiarmid. *University of Maryland School of Medicine, Baltimore, MD.*

Injuries from blasts and explosions pose the "signature" health threat to service members deployed in recent military conflicts. Beyond acute traumatic injury, they can result in chronic sequela arising from significant wound contamination with foreign debris. Embedded metal fragments may cause local foreign-body tissue effects or, with fragment oxidation *in situ*, increased systemic metal concentrations. These raised systemic metal burdens permit insult to target organs far from the site of traumatic injury. Consequently, the US Department of Veterans Affairs established the Toxic Embedded Fragment Surveillance Center, which conducts the only national program that evaluates such war-injured veterans on a population level, after the first Gulf War in 1991. Several findings from this program will be presented, such as urine metal concentrations and surveillance outcomes, which include both routine clinical measures of metal target organ function and novel early effect biomarkers, in VA cohorts exposed either to depleted uranium or to improvised explosive device (IED) injury during combat deployments.

3266 Small Particles and Small Airways: Large Problems for Deployed Military?

M. J. Falvo, and A. M. Sotolongo. *US Department of Veterans Affairs War Related Illness and Injury Study Centers, East Orange, NJ.* Sponsor: M. Johnson

Particulate air pollution from both natural (e.g., sand and dust) and anthropogenic (e.g., burn pit smoke, industrial pollution) sources is ubiquitous in the deployment environments in Iraq and Afghanistan. This includes fine particulates with an aerodynamic diameter less than 2.5 microns ($PM_{2.5}$), which may penetrate deep within the distal or smaller airways. During and after deployment, military personnel exhibit respiratory symptoms and exertional dyspnea that may be associated with their deployment-related exposures, but to date, this has not been confirmed. Post-deployment lung function testing, such as spirometry, has been performed, though it is generally found to be within normal limits, even among symptomatic personnel. Our research and that of others suggests that traditional lung function testing is insensitive for evaluating the smaller airways, and alternative modalities, such as the forced oscillation technique (FOT), may be more sensitive. Work from our national referral clinic has found considerable small airway dysfunction, as detected by FOT, among symptomatic deployed veterans with otherwise normal lung function. Implications of these findings extend beyond the military and are broadly applicable to those with environmental/occupational and inhalational exposures.

3267 Breaking the Code: Identifying the Long-Term Health Implications of Occupational Jet Fuel Exposure in Air Force Personnel Using Military Occupation Codes

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The US Department of Veterans Affairs (VA) and the US Department of Defense (DoD) work together to address the concerns of service members and veterans regarding the health implications of their military service. While historical exposure concerns have focused on those that are characteristic of deployed environments, more frequently, both DoD and VA are receiving inquiries regarding the potential dangers of routine military duties. Exposure to jet fuels is one of the most commonly experienced, regardless of branch. The purpose of this study was to evaluate the sensitivity of military occupation codes as a surrogate for occupational exposure to jet fuels and to investigate the long-term health effects of such an exposure in veterans who served in the Air Force. Occupation codes were used to define levels of exposure, and associations between occupational exposure to fuels and health outcomes were assessed, especially those representing dysfunction in the respiratory and nervous systems. The health data from VA and DoD databases were linked to ascertain these outcomes. This study (1) demonstrates that occupation codes can be used to estimate relative jet fuel exposure in AF personnel, and (2) precedes a more comprehensive, joint VA-DoD, multidisciplinary investigation into the consequences of occupational exposure to jet fuels during service in all branches of the US military, which may have future implications in the development of policy, preventive measures, and health care guidance.

3268 Review of Inhalation Studies of Alternative Jet Fuels in Rodents for Occupational Exposure Limits

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The DoD has put forth significant effort in evaluating alternatively sourced fuels, including those derived from synthetic, plant- or animal-based, or genetically modified organisms, with the ultimate goal of reducing the use of petroleum-derived fuels. Candidate alternative fuels undergo tests of their physical and chemical properties, as well as operational testing, to ensure that they meet performance specifications. In addition to assuring that new fuels are operationally suitable, it is important to ensure that the newly developed fuels are still considered safe for use by service members and any potential toxicity does not exceed the toxicity of conventional fuels. The US Air Force and Navy have used existing toxicity testing paradigms to establish an evaluation program for alternative fuels, with the goal of developing a comparative toxicity database (to include petroleum-derived jet fuels) that can be used to recommend occupational exposure limits (OELs). A variety of fuels have undergone testing in recent years, including a Synthetic Paraffinic Kerosene (SPK) jet fuel generated using a Fischer-Tropsch (FT) process (FT SPK); hydro-treated renewable jet (HRJ) fuel based on oils extracted from the camelina plant (*Camelina sativa*) or bioderived from Hydroprocessed Esters and Fatty Acids (referred to generally as *HEFA*); biologically produced hydrocarbon liquids, such as the bioderived Gevo Jet Blend Stock fuel (Gevo); and the direct-sugar-to-hydrocarbon process that resulted in farnesane made by Amryis Inc. The toxicity of the various fuels was evaluated under a toxicity test protocol described in the DoD Handbook for Aerospace Fuels Certification (MIL-HDBK-510A). The toxicity tests included sensory and dermal irritation and genotoxicity, as well as the extensive short-term and 90-day inhalation exposures with multiple endpoint analyses (in compliance with DODI 3216.01). A summary of these studies will be presented, as well as a discussion of the future direction for jet fuels toxicity studies.

3269 Cannabidiol Science, Safety, and Societal Impacts: Where Do We Stand?

S. Bobst. *ToxSci Advisors LLC, Houston, TX.*

Current market predictions indicate cannabis-derived cannabidiol (CBD) product markets will be over $22 billion by 2022. Because of large demand and market pressures, several national retailers already selling CBD products to consumers, ignoring current Drug Enforcement Administration (DEA) and US Food and Drug Administration (US FDA) restrictions on cannabis-derived products. US states continue to update cannabis legalization laws to align with CBD product popularity and demands from consumers. Federal restrictions on obtaining and funding cannabis and CBD-related research and commerce have placed significant limitations on the traditional safety and efficacy characterizations of CBD use in consumer products. This session will present current CBD basic and clinical research, describe challenges and opportunities for consumer product safety, as well as development of cannabis-derived medical treatments, and lay out legal, regulatory, and social dilemmas created by the complex landscape of commercialization of cannabis-based products.

3270 An Overview of the Pharmacology, Toxicology, and Popularity of CBD

H. Kamendi. *Rx Remedies Inc., Baltimore, MD.*

Cannabidiol, more commonly known as CBD, has gained ever-accelerating popularity over the last three years due to changes in state and local laws and wide-reaching claims that it prevents inflammation, improves appetite, relieves anxiety, aids sleep, and supports or improves many other medical conditions. Hemp-derived CBD sales are on track to become a $1 billion market by 2020. During this time, pharmaceutical preparations will account for nearly 40% of all products. Complex CBD oil extracts are sold online and in natural food stores. They have made their way into national brick-and-mortar establishments, including convenience stores and pharmacies, where they are sold as dietary supplements. Such widespread availability of CBD oils raises many concerns, including the number of poorly characterized cannabinoids and terpenes present in full spectrum extracts. Little is currently known about many effects, mechanisms, and side effects of CBD, for either short- or long-term use, let alone the mechanisms and side effects of complex CBD mixtures.

3271 Assessing Toxicity, Mechanisms of Action, and Therapeutic Potential of Cannabidiol Using Zebrafish

K. Willett. *University of Mississippi, Oxford, MS.*

Recent clinical trials have supported cannabidiol (CBD; Epidiolex) efficacy in helping control seizures in pharmaco-resistant forms of epilepsy such as Dravet syndrome. Largely outside the clinical realm and federal oversight (in the US), CBD use is being popularized for ailments as diverse as anxiety, acne, arthritis and chronic pain, insomnia, and cancer. Zebrafish as a research organism provide a relatively high-throughput model by which both the efficacy and the potential toxicity of CBD can be assessed. For example, using zebrafish that carry a mutation in the voltage-gated sodium channel Nav1.1 (scn1a) to replicate the human Dravet syndrome, we found significant anti-seizure activity with 1μM CBD after 24 h larval exposure. Despite the significant therapeutic potential of CBD, toxicity assessments, particularly for developmental or chronic exposures, are lacking. To assess both the acute and developmental consequences of CBD, zebrafish were exposed through the larval stage to 0.02 to 0.5μM CBD. Developmental deformities, behavioral analysis, and RNA-seq analysis of the transcriptome are being used to identify potential target organ and toxicity pathways (e.g., liver and PXR/RXR). Furthermore, transgenic cannabinoid receptor null (cnr1-/-, cnr2-/-) fish are used to assess receptor-mediated toxicity and/or therapeutic potential. In zebrafish, important differences in cannabinoid bioavailability were identified—specifically, the bioaccumulation of CBD was significantly higher than Δ-9-tetrahydrocannabinol in zebrafish larvae. Adult fish that were developmentally exposed to CBD showed decreased fecundity, supporting the need to consider the developmental origins of health and disease of CBD. Zebrafish offer a good initial tool to measure safety and toxicity and inform policy considerations related to CBD expanded use.

3272 Cannabidiol: Safety, Monitoring, and Drug-Drug Interactions

T. Gaston. *University of Alabama at Birmingham, Birmingham, AL.*
Sponsor: S. Bobst

In June 2018, US FDA approved the first drug composed of an active ingredient of cannabis—a highly purified oral solution of cannabidiol—and with rescheduling, it is now available for use in clinical practice with an indication to treat refractory seizures associated with two severe childhood onset epilepsy syndromes: Lennox-Gastaut syndrome (LGS) and Dravet syndrome (DS). This talk will focus on safety issues as they apply to this pharmaceutical-grade CBD product. First, a brief review of the pharmacology of CBD as it is relevant to the treatment of seizures will be presented, including proposed mechanisms of action and metabolism. Based on the various actions of CBD on the cytochrome P450 system, this raises several concerns toward possible drug-

drug interactions. Next, the available data in both animal models and humans regarding potential pharmacodynamic and pharmacokinetic drug-drug interactions with CBD will be discussed. CBD has been shown to have a significant interaction with the anti-epileptic drug clobazam in particular, which causes increased sedation and elevated blood levels of clobazam's active metabolite. There has been some controversy regarding if this interaction with clobazam is responsible for the efficacy of CBD in treating seizures, and the data to support and refute this will be presented. Next, adverse effects and special populations will be discussed in regard to CBD treatment; this will largely be drawn from the randomized placebo-controlled trial data. Finally, based upon the noted drug-drug interactions and adverse effects, recommended safety monitoring in patients taking CBD will be discussed, particularly from the drug label of highly purified CBD.

W 3273 Cannabidiol: Pain, Pleasure, Potential, and Problems

D. Fox. *Robson Associates LLC, Lancaster, PA.*

The legalization and use of marijuana (tetrahydrocannabinol: THC) and cannabinoid-based products are increasing rapidly in the United States and other North American countries. Currently, 33 states and the District of Columbia have laws that broadly legalize it in some form, although THC is still classified as a Schedule 1 drug by the DEA. In contrast to THC, the non-psychoactive cannabidiol (CBD) was recently rescheduled as a Schedule 5 drug. The marketing, distribution, and widespread availability of CBD raise serious ethical, legal, forensic, and societal issues. Scientific research and discussions about the beneficial clinical, pharmacological, and psychological effects associated with regulated CBD intake are in a controversial and embryonic state. In addition, the acute and long-term potential adverse toxicological effects of chronic CBD in different aged populations are relatively unexplored. This talk will address the biochemical/cell biological mechanisms of CBD and its use for the treatment/management of clinically definable and relevant issues, focusing on pain management and relief and reduction of opioid use/abuse, and including treatment of post-traumatic stress syndrome and other anxiety disorders, convulsion, inflammation, and insomnia.

W 3274 Cannabidiol Incorporation into Consumer Products in the US: Regulatory Challenges to Commercialization

L. Plunkett. *Integrative Biostrategies LLC, Houston, TX.*

This talk will focus on the regulatory concerns related to cannabidiol (CBD) commercialization and build on the information conveyed by preceding speakers related to the science behind CBD as an ingredient with biological properties that impart desirable effects. The ever-changing regulatory landscape surrounding use of cannabis-related ingredients in consumer goods in the US was further complicated in 2019 by the passage of the Farm Bill, which has legalized and expanded the cultivation of hemp and the production of hemp-derived CBD across the US. This talk will provide an update on the regulatory oversight of CBD as it relates to its incorporation into a wide variety of consumer products (e.g., homeopathic drug products, foods, dietary supplements, beverages, and cosmetics), as well as its production as an ingredient for addition to this wide variety of consumer goods. Both federal and state regulatory bodies are involved in various aspects of CBD product production, distribution, and marketing; this talk will focus on public health and safety concerns related to CBD commercialization. Examples of regulatory actions that have been taken by either the federal government or state governments will be discussed, with these examples taken from the most recent regulatory actions. Examples may include (1) recent US FDA actions to crack down on the use of certain unsupported health claims on consumer products containing CBD; (2) US FDA actions in the area of analytical testing of CBD-containing products and findings that certain products contained "unapproved new drug products"; and (3) actions by States like New York and Maine to seize/embargo consumer products sold as foods (baked goods, beverages, etc.) because CBD is not a legal food additive that has been proven safe for use.

W 3275 Have *In Vitro* Teratogenicity Assessments Come of Age in the Pharmaceutical Industry?

M. McNerney. *Bristol-Myers Squibb Company, New Brunswick, NJ.*

The earliest reference to *in vitro* teratogenicity assessment is now 40 years old, and the intervening history of *in vitro* assays represents unwavering interest in their ability to detect serious developmental toxicity, most recently while minimizing animal usage. In the pharmaceutical industry, such assays are routinely used to screen compounds for liability in early development; of late, *in vitro* methods have been pursued for their potential to defer or replace *in vivo* regulatory studies in rats and rabbits. This Workshop has been convened to review the state-of-the-science and to make recommendations for the path forward. After a brief overview of the history of alternative assays for developmental toxicity, the first speaker will present the experience and perspective of the Dutch Medicines Board with *in vitro* assays, and their place in the 2025 framework to eliminate animal studies. The second speaker will present the Merck experience with rat whole embryo culture and mouse embryonic stem cell tests, including developing and characterizing an integrated prediction model that has been used for routine screening for most of the past decade. The third speaker will contribute the perspective of a Center for Drug Evaluation and Research reviewer on the utility of *in vitro* teratogenicity assays. The fourth speaker will discuss data collected and analyzed by the DruSafe/IQ Consortium, comparing findings from 90 compounds that were tested both *in vitro* and *in vivo*. Importantly, this session will segue from presentations into discussion/Q&A among audience members and speakers, with the expectation that the contributions of many in the audience (whether experts, novices, or data consumers) will lead to a fuller and more nuanced understanding of the ways that such assays can be successfully employed.

W 3277 Alternative Methods for Developmental Toxicity Testing of Pharmaceuticals: An EU Regulatory Point of View

P. Theunissen. *CBG-MEB, Utrecht, Netherlands.* Sponsor: M. McNerney

In the current regulatory framework, embryo-fetal developmental toxicity (EFDT) testing is primarily performed in two species. Recently, the need of testing EFDT in two species and the reproducibility of these EFDT studies have been questioned. Alternative assay strategies for EFDT testing can be a (partial) substitute for animal data, but currently they are only sporadically provided as evidence for first-in-human trials and marketing authorization applications (MAA). In the (drafted) revision of the ICH S5(R3) Guideline, for the first time alternative testing strategies are proposed as an approach for addressing EFDT risk. Upon adoption, it is expected that alternative testing strategies will be increasingly provided for regulatory qualification and, subsequently, at MAA. In addition, it is expected that within predefined contexts of use, including a known mode of action, within class potency ranking, and for certain indications, alternative assays for EFD toxicity testing will provide sufficient scientific evidence for regulatory decisions, resulting in more mechanistically driven decisions and reduced animal testing. However, how will these data contribute to risk assessment and labeling? Current European use of animal data and experience with alternative assays for EFD toxicity testing for risk assessment and labeling will be discussed in perspective of the expected paradigm shift by ICH S5(R3).

W 3278 Putting Alternative Developmental Toxicity Assays to Work

K. Brannen. *Merck & Co. Inc., West Point, PA.*

The scientific community has sought predictive alternatives to embryo-fetal development (EFD) studies for decades, but translating the concept into reality has presented challenges. Determining whether such an assays will actually improve drug discovery and development has been even more complicated. This presentation will use one pharmaceutical company's experience designing, characterizing, and using two *in vitro* assays as an example to illustrate the potential utility of these screens individually and in combination. Rat whole embryo culture (WEC) and a mouse transcriptional embryonic stem cell test (mTEST) were evaluated individually and in combination. These assays were refined to maximize sensitivity for detecting developmental toxicants (specifically molecules that induce malformations and/or embryo-fetal lethality), and an integrated model was developed that incorporated results from both assays and provided an exposure-based prediction based on the WEC results. The assays and the associated exposure-based prediction model were initially tested with more than 50 compounds with known *in vivo* outcomes, and in the almost 10 years since, they have been used routinely in discovery programs to screen for developmental toxicity potential and determine timing of *in vivo* studies. Based on an analysis of 83 compounds with known *in vivo* outcomes (Green et al. 2018, *Appl in vitro Toxicol* 4(1):44-53), sensitivity and specificity for the WEC model alone was 78% and 78%, respectively, and 64% and 57% for the mTEST prediction model. The integrated prediction model provided improved sensitivity of 89% compared with either assay individually, but the resulting specificity was 54%. Case examples of discovery compounds that were tested in WEC and mTEST and subsequently evaluated in preliminary and/or definitive EFD studies will be presented. Implications of

assay and prediction model designs will be discussed, and several questions that remain to be answered will be raised to spur discussion with the panel and audience.

3279 The Role of Alternative Assays in Informing DART Risk Assessment for Pharmaceuticals

R. Wange. *US FDA/CDER, Silver Spring, MD.*

In opening up the ICH S5 guidance for revision, the participating drug regulatory authorities and regulated industry committed to providing guidance on the attributes that alternative assays should have, if they are to be used for regulatory purposes. In addition, per the Concept Paper, the guidance is to identify the circumstances under which results from alternative assays would be considered to be sufficiently informative as to permit regulatory decision-making without negatively impacting trial subject or patient safety. As currently drafted, the revised guidance indicates that each region will independently assess whether a submitted alternative assay is qualified for the intended context of use. This presentation will discuss the current approach of CDER to conducting qualification assessments for DART alternative assays dossiers, as well as presenting an analysis of our experience to date with the use of alternative assays in IND, NDA, and BLA submissions, focusing on the types of assays received and whether the assays have had any impact on the nonclinical or clinical development programs.

3280 Concordance between *In Vitro* Teratogenicity Assessments and *In Vivo* Findings: The IQ DruSafe Assessment

M. McNerney. *Bristol-Myers Squibb Company, New Brunswick, NJ.*

IQ DruSafe established a working group to evaluate concordance of three *in vitro* assays with findings reported from rat or rabbit EFD studies. Eight companies contributed 90 compounds to a database; each entry has at least one *in vitro* and one *in vivo* pair. *In vivo* findings with malformations or mortality were deemed positive; otherwise, they were considered negative. Too, each company was asked to indicate whether its *in vitro* assay data predicted positive or negative *in vivo* findings. First-tier queries examined whether a given assay predicted that one or both species was positive or negative, with subsequent analyses by species and type of toxicity. Acceptable Sensitivity and Specificity were set at 70%. Positive and negative predictive values (PPV and NPV) were adjusted for the companies' portfolio prevalence of 30%; likelihood ratios (LR) also were calculated. Acceptable rates of false negatives were set at = 10%. Fifty-six compounds had zebrafish embryo culture data. Apical analyses yielded 68% sensitivity, 32% specificity, 30% PPV, and 69% NPV; LR for PPV and NPV were each ~1. Individual analyses (by toxicity type or species) were not further optimized. The overall rate of false negatives for this assay was 21.4% and did not improve by further 2x2 analyses. Murine embryonic stem cell data were available for 48 compounds (including 47 rat and 32 rabbit studies). Apical analyses yielded 72% sensitivity, 37% specificity, 33% PPV, and 76% NPV. The LR for PPV and NPV were ~1. The apical rate of false-negative predictions for embryonic stem cells was 16.6%. Subsequent 2x2 analyses yielded similar results. Rat whole embryo culture data were available for 29 compounds (including 29 rat and 20 rabbit studies). Apical analyses yielded 82% sensitivity, 68% specificity, 51% PPV, and 90% NPV. The LR for PPV and NPV were ~2.5 and 3.8, respectively. LR for NPV were invariably improved with subsequent 2x2 analyses, the most significant of which was the assessment of rat malformations or mortality (LR ~9.3). The apical rate of false negatives was 10.3%, and most 2x2 assessments were at least equivalent (exception: the rate of false negatives for rat or rabbit mortality was 34.5%). The limitations and implications of these findings will be discussed.

3281 Immune-Mediated Adverse Drug Reactions: State-of-the-Art Learnings from Preclinical and Clinical Drug Development

A. Sharma. *Genentech Inc., San Francisco, CA.*

Adverse drug reactions (ADR) are a major cause for attrition resulting in termination of drug development, denied commercialization, market withdrawal, or restricted prescribing of new pharmaceuticals. Some ADRs are considered predictable as the injury results from excessive pharmacology at the intended target of the drug. The term *idiosyncratic* is assigned to ADRs where injury is unrelated to drug's mechanism of action and often immune mediated in nature. Immune mediated adverse drug reactions (IM-ADR) represent less than 20% of all ADRs, but they can be severe in clinical terms and have the poorest concordance between man and preclinical species. These are usually not detected until late in development, after significant investment in development and after large numbers of patients have been put at risk. Affected patients may present with fever, rash, hematologic abnormalities, or damage to a variety of internal organs. IM-ADRs can be associated with small molecule-based drugs and are characterized by activation of drug-antigen specific T and B cells of the acquired immune system. Reactive metabolites covalently bind to proteins that are processed to form neoantigens. Alternatively, they establish noncovalent interactions on class I or II MHC molecules that lead to immunogenic complexes. Large molecule drugs (e.g., monoclonal antibodies) also represent a unique type of risk. Recent clinical reports have highlighted the possibility of increased severity and incidence of IM-ADRs for specific immune checkpoint inhibitor monoclonal antibodies when given in combination with small molecule drugs or with other antibody-based immune checkpoint inhibitors. Despite much progress, understanding of the underlying mechanisms of IM-ADRs is incomplete, and qualified tools for risk assessment are lacking. The goals of this discussion are (1) to bring together in one session the IM-ADR-related preclinical and clinical development learnings for both small and large molecule drugs as an attempt to identify synergies between these modalities, and (2) to discuss the state-of-the-art models as well as future approaches and biomarkers that will enable improved prediction and prevention of IM-ADRs. The first presentation will review the chemistry of small molecule-based drug antigens and the molecular basis of IM-ADRs mediated by the adaptive immune system. The second presentation will discuss the role of drug metabolism and the immune biology of skin, a frequent target organ of IM-ADRs. The third presentation will review the role of the innate immune system in IM-ADRs. The fourth presentation will illustrate how humanized preclinical animal models can advance our mechanistic understanding of IM-ADRs. The fifth presentation will shift the focus of the session to large molecule-based drugs and present data obtained from patients experiencing IM-ADRs. The final presentation will discuss preclinical models used to predict the immunogenicity of large molecule-based drugs. All speakers will emphasize what are the tools currently available for improving risk assessment to enable the development of safer drugs.

3282 The Chemical Basis of Drug Hypersensitivity and Development of *In Vitro* Assays to Predict Intrinsic Immunogenicity

D. J. Naisbitt. *University of Liverpool, Liverpool, United Kingdom*. Sponsor: A. Sharma

T cell-mediated drug hypersensitivity reactions represent a major impediment to the drug development process, as they are currently impossible to predict. Susceptibility is dependent on three critical factors: (1) exposure to the drug antigen in a relevant form, (2) the availability of a T cell repertoire for the drug antigen, and (3) the availability of HLA molecules for drug antigen binding. It most be noted, however, that most individuals who express all three factors don't develop hypersensitivity when exposed to drugs. This is because immune regulatory pathways also contribute to susceptibility through controlling the strength, nature, and duration of the immune response. Since expression and activity of these pathways are determined by the genetic makeup of the individual, alongside environmental factors and disease, the outcome of drug exposure, be it a health benefit or hypersensitivity, is currently impossible to predict. This presentation will review the chemical basis of drug hypersensitivity and how immune regulatory pathways impact the development of a drug-specific T cell response. The discovery of strong associations between expression of single HLA alleles and susceptibility to specific forms of drug hypersensitivity and the development of T cell priming assays have allowed researchers to show that certain drugs bind selectively to HLA risk alleles to activate T cells. However, using currently available T cell priming assays, it is not possible to predict the intrinsic immunogenicity of new drugs and chemicals. Thus, the second component of the presentation will detail how we are developing a stepwise screening approach for drug immunogenicity assessment using lymphocytes from HLA-typed healthy donors.

3283 Bioactivation of Drugs in the Skin: Examining the Concepts of Necessity and Sufficiency in Drug-Induced Dermal Toxicity through Metabolism, Immunology, and Host-Dependent Factors

A. M. Sharma. *Genentech Inc., San Francisco, CA.*

Advances in the understanding of drug-induced skin toxicities have been slow given the skin's complex anatomical nature. Current advances in the study of cutaneous adverse drug reactions are attributable to recent developments in our ability to dissect individual layers of skin and study discrete components. This has led to a deep appreciation for the skin as both a metabolically and immunologically competent organ. It is now understood that

the skin not only serves as a protective barrier with limited drug biotransformation ability, but also possesses high immune activity/function, capable of myriad responses. While the immune response of the skin to drugs is vastly different from that of the liver due to evolutionary conditioning, it frequently occurs in response to various drug classes and manifests as a spectrum of hypersensitivity reactions. Association of other factors such as human leukocyte antigen (HLA) polymorphisms may play a significant role for particular drugs. This presentation aims to introduce the concept of what is necessary in causing adverse skin reactions from what may be sufficient. Emerging findings will be translated into proposed mechanisms of drug metabolism and immunity in the skin that are likely responsible for rashes and other local allergic responses. These unique biological aspects of the skin, and their translation into implications for drug development and the use of animal models, will be discussed. The concept of necessity versus sufficiency in drug-induced skin toxicity is an emerging topic of high interest to both academics and drug developers alike.

3284 Role of the Innate Immune System in Adaptive Immune Mediated Idiosyncratic Drug Reactions

J. Uetrecht. *University of Toronto, Toronto, Canada.*

Idiosyncratic drug reactions (IDRs) represent a significant source of morbidity and mortality and significantly increase the risk of drug development. An accurate method to predict the risk that a drug candidate would cause IDRs would be a major advance. There are multiple lines of evidence that most IDRs are immune mediated. The most severe IDRs appear to be due to cytotoxic T cells, but antibody-mediated reactions also are significant. Most people do not have a serious IDR to culprit drugs and the major response, especially in the liver, appears to be immune tolerance. An adaptive immune response depends on the recognition of antigens by a combination of HLA on antigen presenting cells and T cell receptors. The HLA genotype is inherited, but even if a patient has the HLA genotype associated with a specific IDR, it is unlikely they will have an IDR to that drug. Presumably they lack T cells with the required T cell receptor. The T cell receptor repertoire is produced by recombination of genes, and it is shaped by everything that an individual has been exposed to. The generation of an adaptive immune response requires an innate immune response. The innate immune response can vary between species and between individuals in a species, but it is much less idiosyncratic. For example, clozapine-induced agranulocytosis does not occur in most patients, but most patients do have a transient innate immune response with increases in IL-6, C-reactive protein, and a paradoxical neutrophilia. We have shown that clozapine also induced an innate immune response in rats. Clozapine also leads to an activation of inflammasomes. Olanzapine, which is very similar to clozapine and forms a reactive metabolite, does not cause an innate immune response in rats and does not activate inflammasomes. Penicillamine causes a variety of autoimmune reactions in humans and in Brown Norway rats. About 50% of rats develop the autoimmune syndrome, with a transient spike in serum IL-6 at 24 hours that is back to baseline in three days. The height of the transient IL-6 spike predicts which animals develop autoimmunity three weeks later. It is plausible that testing the ability of drug candidates to induce an early innate immune response, first in animals and later in humans, will predict which drug candidates are likely to cause serious IDRs. New data will be presented that tests this hypothesis.

3285 Animal Models for HLA-Linked Adverse Drug Reactions

M. Norcross. *US FDA/CDER, Silver Spring, MD.* Sponsor: A. Sharma

Adverse drug reactions (ADRs) are a major obstacle to drug development, and some of these, including hypersensitivity reactions to the HIV reverse transcriptase inhibitor abacavir (ABC), are associated with HLA alleles, particularly HLA-B*57:01. However, not all HLA-B*57:01+ patients develop ADRs, suggesting that in addition to the HLA genetic risk, other factors may influence the outcome of the response to the drug. Animal models that express the HLAs that are linked to drug reactions could provide *in vivo* systems to study both genetic and host factors that control adverse reactions. To study HLA-linked ADRs *in vivo*, we generated HLA-B*57:01-Tg mice and show that, although ABC activated Tg mouse CD8+ T cells *in vitro* in a HLA-B*57:01-dependent manner, the drug was tolerated *in vivo*. In immunocompetent Tg animals, ABC induced CD8+ T cells with an anergy-like phenotype that did not lead to ADRs. In contrast, *in vivo* depletion of CD4+ T cells prior to ABC administration enhanced DC maturation to induce systemic ABC-reactive CD8+ T cells with an effector-like and skin-homing phenotype along with CD8+ infiltration and inflammation in drug-sensitized skin. B7 costimulatory molecule blockade prevented CD8+ T cell activation. These HLA Tg mice provide a model for ABC tolerance and for the generation of HLA-B*57:01-restricted, ABC-reactive CD8+ T cells dependent on both HLA genetic risk and immunoregulatory host factors.

3286 Understanding Autoimmunopathies from Immunotherapy: Biomarkers and Mechanisms

J. Balko. *Vanderbilt University Medical Center, Nashville, TN.* Sponsor: A. Sharma

Immune checkpoint inhibitors induce durable antitumor responses across cancer types but may simultaneously unleash autoimmune-like toxicities affecting nearly all organs. The molecular underpinnings of these toxicities have not been well characterized but likely vary with specific immunotherapies and sites of toxicity. T cell receptor sequencing (TCRseq) performed on sites of fulminant or aggressive immunopathies (e.g., myocarditis and encephalitis) has demonstrated highly oligoclonal T cell repertoires that may hint at antigens from which peripheral tolerance has been released, or that mimic tumor-specific antigens associated with immunologic responsiveness to therapy. Preclinical and anecdotal clinical data suggest that therapeutic responsiveness can be uncoupled from checkpoint inhibitor-induced autoimmunity via inhibition of pathways such as type-I interferon or TNFa, although this has yet to be demonstrated in controlled trials. This talk will focus on current findings surrounding the molecular features and biomarkers associated with distinct autoimmunopathies as well as strategies to improve risk:benefit ratios in the treatment of cancer with immune checkpoint inhibitors.

3287 Immune-Mediated Adverse Events in Preclinical Toxicology Species: Role of Immune Complexes Formed from Therapeutic Protein-Antibody Complexes

V. Jawa. *Merck & Co. Inc., Kenilworth, NJ.* Sponsor: A. Sharma

The immune response to human therapeutic proteins (TP) in preclinical species is often excessive and can lead to immune complex mediated adverse events like hypersensitivity. These reactions need to differentiate pharmacology from an immunogenicity mediated event, especially when the pharmacology could be further modified by the engagement of immune modulatory receptors on immune cells. Some accompanying events associated with immunogenicity would be presence of anti-drug antibodies and loss of exposure due to accelerated clearance based on kinetics of immune response. Based on their size and composition of TP: anti-therapeutic antibodies (ADA), the immune complexes can deposit in the vasculature or kidneys, leading to vasculitis or renal pathology. This talk will propose strategies for a proactive risk assessment strategy and design of the relevant assays to support a proactive assessment of immunogenicity mediated adverse events.

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